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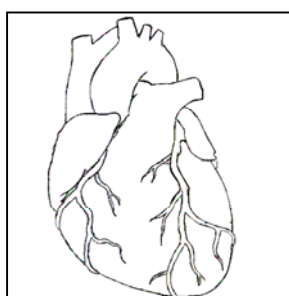
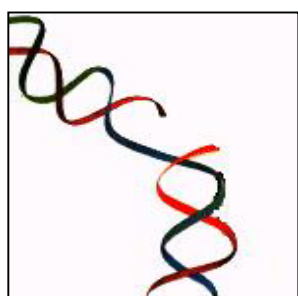
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# ALCOHOL DRINKING PATTERNS

Genetic predictors and associations with  
coronary heart disease, obesity and mortality



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May 2006

# ALCOHOL DRINKING PATTERNS

Genetic predictors and associations with  
coronary heart disease, obesity and mortality

PhD thesis

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## THIS THESIS IS BASED ON FOUR PUBLICATIONS:

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### Paper 1

Tolstrup JS, Nordestgaard BG, Rasmussen S, Tybjaerg-Hansen A and Grønbæk M:  
Alcoholism and drinking habits predicted from alcohol dehydrogenase genes. *Submitted*.

### Paper 2

Tolstrup JS, Jensen MK, Tjønneland A, Overvad K, Mukamal KJ and Grønbæk M:  
A prospective study of alcohol drinking pattern and coronary heart disease in women and men. *British Medical Journal* 2006; 332: 1244-8.

### Paper 3

Tolstrup JS, Heitmann BL, Tjønneland AM, Overvad K, Sørensen TIA, Grønbæk M:  
The relation between drinking pattern and body mass index, waist and hip circumference. *International Journal of Obesity* 2005; 29: 490-97

### Paper 4

Tolstrup JS, Jensen MK, Tjønneland A, Overvad K, Grønbæk M:  
Drinking pattern and mortality in middle-aged men and women. *Addiction* 2004; 99, 324-30

## P R E F A C E

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Janne Schurmann Tolstrup, May 2006.



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## PAPERS

- 1 Alcoholism and drinking habits predicted from alcohol dehydrogenase genes
- 2 A prospective study of alcohol drinking pattern and coronary heart disease in women and men
- 3 The relation between drinking pattern and body mass index, waist and hip circumference
- 4 Drinking pattern and mortality in middle-aged men and women





## INTRODUCTION

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Alcohol is used worldwide, as a legal drug by some, and as a natural part of the diet by others. The World Health Organization estimates that only five percent of the adult Danish population abstain from alcohol drinking.<sup>1</sup> On the individual level, drinking behaviour is influenced by environmental factors, such as culture and religion, and by heredity; for instance, twin studies have shown that approximately 50 percent of problem drinking and alcoholism can be explained by heritability.<sup>2,3</sup> The contribution of specific genes in explaining drinking behaviour is sparsely studied, especially among Caucasians.

People who have experienced the malaise following an episode of heavy drinking may find it intuitively true that drinking pattern is important when studying risks associated with alcohol. Nevertheless, the majority of epidemiologic studies are based on a single measure summarising alcohol exposure into an average amount. Recently, however, evidence has emerged that this one-dimensional approach does not adequately account for health risks associated with alcohol drinking; important variation is comprised in the *drinking pattern*. In studies that focus on amount only, individuals who drink relatively small amounts on a number of drinking sessions are categorised with individuals consuming the same weekly amount of alcohol on one Saturday night. These two patterns of drinking may be associated with very different risks.

Drinking pattern is not unambiguously defined and has been characterised as drinking with meals, in weekends only, to intoxication, to a certain blood alcohol level, more than a certain amount per session (6 drinks, 13 drinks, ½ a bottle of spirits, etc.), and amount and frequency have been combined.<sup>4,9</sup> A common feature of these approaches is that alcohol exposure is described in more than one dimension. Only few studies have sought to clarify the relative roles of amount and frequency of alcohol intake.<sup>10</sup>

Hypotheses tested in the present thesis relate to genetic predictors of drinking patterns and alcoholism, and to the influence of drinking patterns on different health endpoints. In *Study 1*, functional variation in main alcohol degrading enzymes is associated with the individual's drinking behaviour, such as weekly amount of alcohol intake and daily drinking, and with risk of alcoholism. In *Studies 2 to 4*, associations between drinking frequency and coronary heart disease, obesity and mortality, are examined to study if drinking frequency has independent effects on the different endpoints. Thus, in these studies, the main focus is not on studying differences between nondrinkers and drinkers, but rather on studying drinkers characterised by different drinking patterns.

The present thesis is structured as follows: First, background and specific aims of the thesis are presented, followed by a brief outline of the data sources on which it is based. Thereafter, results from Studies 1 to 4 are summarised, which is followed by discussions of results and of potential biases. Finally, main conclusions are presented and future perspectives are discussed.

## BACKGROUND AND AIMS

### Genetic predictors of alcohol drinking patterns and alcoholism

Whole genome screens have demonstrated linkage between phenotypes for problem drinking and chromosome 4, more precisely in the region of the alcohol dehydrogenase (*ADH*) gene cluster.<sup>11</sup> This region consists of seven loci that encode alcohol degrading enzymes (Figure 1).<sup>12</sup> *ADH1*, *ADH2* and *ADH3* are characterised by enzyme products mainly responsible for degrading ethanol (subsequently referred to as alcohol), while other *ADH* enzymes mainly degrade other types of alcohols.

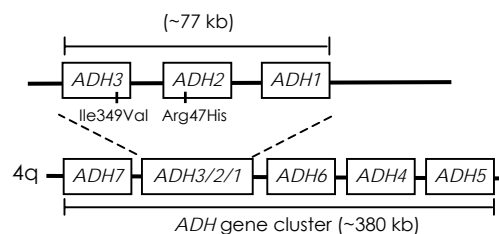


FIGURE 1. Map of the human alcohol dehydrogenase genes (*ADH*s). A detailed map of *ADH1*, *ADH2* and *ADH3* is expanded above the map of the gene cluster as a whole. Genetic variation affecting enzyme activity is found in *ADH2* and *ADH3* (the resulting amino acid changes are indicated). Reproduced from Osier 2002.<sup>12</sup>

Alcohol is passively absorbed from the stomach and duodenum and is distributed within the body's water compartment. The rate of alcohol degradation is 30 to 90 minutes per drink and is influenced by sex, frequency of alcohol intake, age and genetic factors.<sup>13</sup> Degradation of alcohol into acetaldehyde is rate limiting for the reaction (Figure 2). Acetaldehyde is normally rapidly degraded to acetate and water by acetaldehyde dehydrogenase (*ALDH*).



FIGURE 2. Alcohol degradation. *ADH*; alcohol dehydrogenase, *ALDH*; acetaldehyde dehydrogenase.

Genetic variation with functional implications is found at *ADH2* and *ADH3* loci. At *ADH2*, alleles *ADH2·2* and *ADH2·1* produce enzymes with a 38 fold difference in *in vitro* alcohol degradation rate, and at *ADH3*, alleles *ADH3·1* and *ADH3·2* produce enzymes with a 2.5 fold difference.<sup>14</sup>

During normal alcohol degradation, the blood level of acetaldehyde is low. When concentrations of acetaldehyde become high, e. g. during treatment with disulfiram (Antabuse®) or in individuals with defective *ALDH* (not found in Caucasians<sup>15</sup>), individuals experience severe nausea and flushing and abstain from drinking alcohol. It is possible that individuals carrying the slow alcohol degradation *ADH2·1* and *ADH3·2* alleles are able to drink alcohol without experiencing discomfort due to elevated

acetaldehyde levels. If so, these individuals may be more likely to drink alcohol in larger amounts and more often, and may be at a higher risk of developing alcoholism compared with individuals carrying the fast alcohol degradation *ADH2*:2 and *ADH3*:1 alleles. This is only sparsely studied in Caucasians and only in relatively small case-control studies.

The purpose of Study 1 was to test the hypothesis that individuals with *ADH2* and *ADH3* slow compared with fast alcohol degradation drink more alcohol and more often, and are at higher risk of alcoholism.

#### Alcohol drinking pattern and coronary heart disease

Substantial epidemiological evidence suggests that alcohol has beneficial effects on the cardiovascular system.<sup>16-18</sup> Plausible mediating factors such as increased high-density lipoprotein levels, lower plasma fibrinogen levels and reduced platelet aggregation have also been identified.<sup>19</sup> However, important questions still remain. Among these is the role of drinking pattern, especially among women, who differ from men in both alcohol pharmacokinetics and in absolute risk of coronary heart disease. An episodic drinking pattern with large consumptions of alcohol per drinking session (*binge drinking*) has been associated with a higher risk of coronary heart disease,<sup>6,7,9,20</sup> but few studies have sought to clarify the relative roles of amount and frequency of alcohol intake.

In Study 2, associations between amount and frequency of alcohol intake and risk of coronary heart disease are examined. The main purpose was to test the hypothesis that the risk of coronary heart disease is lower among individuals drinking frequently compared with individuals drinking less frequently for the same weekly amount of alcohol intake

#### Alcohol drinking pattern and obesity

Alcohol intake may be associated with obesity for several reasons; alcoholic beverages are energy dense and are generally not substituting food but rather added to the total energy intake.<sup>21</sup> Furthermore, metabolites from alcohol degradation may inhibit fat oxidation.<sup>22</sup> However, research in this area remains inconclusive, with some studies showing amount of alcohol to be positively associated with obesity,<sup>23-25</sup> others showing no association<sup>26</sup> and still others showing an inverse association.<sup>27</sup> Little is known about the relationship between alcohol drinking pattern and obesity. Obese individuals are at increased risk of coronary heart disease and any association between drinking pattern and obesity could hypothetically explain part of an association between drinking pattern and coronary heart disease.<sup>28-31</sup>

The main purposes of Study 3 were to 1) examine the association between alcohol drinking frequency and general obesity (measured as body mass index) and 2) examine the association between alcohol drinking frequency and fat distribution (measured as waist and hip circumference).

#### Alcohol drinking pattern and all-cause mortality

A J-shaped association between amount of alcohol intake and all-cause mortality has been found in many prospective studies.<sup>32-35</sup> This is thought to reflect a reduced risk of coronary heart disease among light to moderate drinkers, and an increased risk of

conditions like liver cirrhosis, chronic pancreatitis, cancers and injuries among more heavy drinking individuals. For public health purposes, this association is important because it reflects net loss of life attributable to alcohol consumption and thus constitutes the scientific basis for creating guidelines on sensible drinking. Other studies have found that a binge-like drinking pattern is associated with a higher risk of mortality, but to our knowledge, no studies have sought examine if the J-shaped curve between alcohol and all-cause mortality depends upon the alcohol drinking frequency.<sup>4,6,36</sup>

In Study 4, the main purpose was to test the hypothesis that the J-shaped association between amount of alcohol intake and mortality is modified by drinking frequency, so that for each level of weekly amount, the risk of mortality is higher among individuals with a non-frequent intake compared with individuals with a more frequent intake.

## DATA SOURCES AND ASSESMENT OF MEASUREMENTS

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### The Copenhagen City Heart Studies (data source for Study 1)

In 1976, a random sample of the Danish general population above 20 years living in the Copenhagen area was invited to participate in the Copenhagen City Heart Study (number of participants 14,223; response rate 74%). This examination was followed by three more examinations; a second examination in 1981-83, where all previously invited plus 500 new individuals aged 20-24 years were invited (number of participants 12,698; response rate 70%); a third examination in 1991-94 where all previously invited plus 3000 new individuals aged 20-49 years were invited (number of participants 10,135; response rate 61%); and a fourth examination in 2001-03 where all previously invited plus an additional sample of 1040 individuals aged 20 to 29 years were invited (number of participants 6,238; response rate 50%). All participants gave informed consent and the ethics committee for Copenhagen and Frederiksberg approved the study (100.2039/91).

Before visiting the study clinic, participants completed a questionnaire (including questions on alcohol intake). At the clinic visit, physical examinations were performed and questionnaires were checked for missing information and any uncertainties were clarified. More particularly, blood samples were taken for DNA purification in 1991-94 and thus individuals participating in this examination constitute the study sample for Study 1. Enrolment and examination procedures have been described in more detail elsewhere.<sup>37,38</sup>

*Ethnicity:* Distributions of *ADH2* and *ADH3* genotypes vary considerably according to ethnicity,<sup>12</sup> and ethnicity is likely to be associated with alcohol drinking patterns (*population stratification*). Hence, knowledge of the ethnic composition of the study population is essential. Eligibility criterion for participation in any of the examinations was Danish citizenship and therefore, the Copenhagen City Heart Study does not reflect the ethnic admixture of Copenhagen (the proportion of inhabitants with foreign citizenship was eight percent in 1994).<sup>39</sup> However, even a few participants of foreign ethnicity could potentially confound our results since the fast alcohol degradation *ADH2*·2 allele is rare among Caucasians but frequent in other populations. Information on ethnicity was not assessed at the examinations, and hence information on birthplace was obtained from the Civil Registration System. Participants born in Asia, Africa, the Middle East, South America or Greenland were excluded from further study (n=211).

### The Diet, Cancer and Health Study (data source for Studies 2, 3 and 4)

During 1993 to 1997, a random sample of Danish men and women aged 50 to 65 years living in the Copenhagen and Aarhus areas were invited to participate in the Diet, Cancer and Health Study (number of participants 57,053; response rate 35%). All participants gave informed consent and the ethics committee for Copenhagen and Frederiksberg approved the study (KF 01-116-96).

Eligible cohort members were born in Denmark and had no previous cancers at the time of inclusion. Participants completed a food-frequency questionnaire (including questions on amount of alcohol intake) before visiting a study clinic, where another

questionnaire concerning lifestyle factors (including questions on alcohol drinking frequency) was completed. Trained personnel checked for missing information and clarified uncertainties in the questionnaires with every participant. Enrolment and examination procedures have been described in detail elsewhere.<sup>40</sup> A description of the development and validation of the food frequency questionnaire has been published previously.<sup>41,42</sup>

#### Assessment of alcohol exposures by frequency questionnaires

Information on amount of alcohol was obtained by frequency questionnaires in the Copenhagen City Heart Study and in the Diet, Cancer and Health Study. The validity of this method has been examined in the Danish part of the MONICA project.<sup>43,44</sup> Here, information on alcohol intake obtained by frequency questionnaire was compared with information on alcohol intake obtained by dietary interview. A close agreement between the two information sources was observed. Although this comparison does not represent a true validation of frequency questionnaires, dietary interviews are considered to convey more accurate information than frequency questionnaires. Dietary interviews are time consuming and expensive, and assessing information by this method in large cohorts such as the Copenhagen City Heart Study and the Diet, Cancer and Health Study would not be feasible.

It would be informative to validate selfreported alcohol intake against a biochemical marker, because it is a more objective measure and potential errors of self-reports and markers are unlikely to be correlated. There is no perfect biochemical marker of alcohol intake, but the level of high-density lipoprotein cholesterol in the blood has been suggested.<sup>45,46</sup> In the Copenhagen City Heart Study, a dose-response relation between alcohol and high-density lipoprotein cholesterol has been observed by others, also speaking in favour of the validity of assessing alcohol intake by frequency questionnaires.<sup>47</sup> The validity of obtaining information on drinking frequency from questionnaire has not been examined.

#### Assessment of endpoints by linkage with national registers

Participants were followed by linkage with central Danish registries using the unique person identification number. The Danish Hospital Discharge Register<sup>48</sup> and the Danish Register of Causes of Death<sup>49</sup> contain information on all admissions to Danish hospitals and causes of death, respectively. Diagnoses are classified according to the World Health Organization's *International Classification of Diseases*, using the eighth revision until 1994 and the tenth revision from 1994 and onward. Advantages of assessing endpoints from central registers include the ease by which large study populations can be followed continuously for various endpoints, and that loss to followup is almost negligible (in the present studies, less than one percent). For comparison, studies like the Health Professionals Follow-up Study in the USA have to rely on selfreport, which is more time-consuming and generally implies more loss to followup. By combining data from the Danish Hospital Discharge Register and the Danish Register of Causes of Death, information on endpoints (alcoholism and coronary heart disease) was assessed, defining a case as either hospital admission with the respective endpoint as the primary or secondary diagnosis, or the respective endpoint as the cause or contributing cause of

death. Admissions coded by ICD-8 modification codes 1 ('Observational') and 2 ('Not found') were excluded. Modification codes are not used in the ICD-10 system.

The diagnosis of coronary heart disease which consists of myocardial infarction and stable and unstable angina pectoris, has not been validated as an entity. With respect to myocardial infarction, the validity of the ICD-8 diagnosis has been analysed by others. Thus, 94 percent of myocardial infarction diagnoses in the Danish Hospital Discharge Register and the Danish Register of Causes of Death were later confirmed in the DANMONICA study.<sup>50</sup> The diagnostic sensitivity for myocardial infarction in that study was 78 percent. The diagnosis of alcoholism has not been validated.

Information on vital status was obtained from the Civil Registration System and this information is considered to convey almost perfect sensitivity and specificity.<sup>51</sup> Information on deaths is registered with a delay of approximately four days or one month after the incident, depending on whether the person died in Denmark or abroad.

#### Statistics for thesis

(For other statistics, please refer to Papers 1 to 4)

*Calculation of odds ratios for alcoholism and confidence intervals from previous studies of ADH2 and ADH3 (Table 1):* In order to compare our results on ADH genotypes and alcoholism with results of others, we calculated odds ratios on the basis of presented results in previous studies. Sample sizes of some of these data were small and hence usual asymptotic methods are unreliable. Thus, exact logistic regression was applied<sup>52</sup> (proc logistic with the exact statement invoked [SAS 8.2]). Pooled odds ratios were estimated by logistic regression, applying fixed effects for ADH2 and ADH3 genotypes, and random effects to account for between-study heterogeneity (proc nlmixed [SAS 8.2]).

*Sensitivity analysis of misclassifications of coronary heart disease (Table 4):* In order to evaluate the impact of possible misclassification of coronary heart disease diagnoses, sensitivity analyses were performed. Different combinations of false positive rates (Fpr) and sensitivity (Se) were assumed, and corrected incidence rates of coronary heart disease were calculated by applying the following equation:<sup>53</sup>

$$A' = Se \cdot A + Fpr \cdot T,$$

where  $A'$  is the number of participants classified with coronary heart disease,  $A$  is the true number of participants with disease, and  $T$  is the true person time at risk. Assuming that false-negatives are adding negligible person time,  $T \approx T'$ , where  $T'$  is the observed person time, and hence:

$$A = (A' - Fpr \cdot T') / Se \Rightarrow A/T' = (A'/T' - Fpr) / Se,$$

where  $A/T'$  is the corrected incidence rate in the respective category. Corrected incidence rate ratios were subsequently calculated by dividing corrected incidence rates in exposed categories with the corrected incidence rate in the reference category.

*Comparison of participants and nonparticipants among those invited (Figure 9):* In order to compare rates of all-cause mortality, alcoholism and alcoholic liver cirrhosis among participants and nonparticipants of the 1991-94 examination of the Copenhagen City Heart Study, risk estimates were computed by means of Cox proportional hazard regression. Information on vital status was obtained from the Civil Registration System, and information on alcoholism and alcoholic liver cirrhosis was obtained from the Danish

Hospital Discharge Register. In the Cox model, age was used as the time scale and analyses were corrected for delayed entry and adjusted for sex. The followup time for each individual was the period from date of the 1991-94 examination of the Copenhagen City Heart Study until date of the respective endpoint, death, emigration, or January 1, 2004, whichever came first. Data to perform similar analyses for participants of the Diet, Cancer and Health Study were not available.



## SUMMARY OF RESULTS

### Study 1: *ADH2* and *ADH3*, alcohol drinking patterns and alcoholism

Among the 9080 participants from the Copenhagen City Heart Study who were eligible for this study, allele frequencies coding for slow alcohol degradation were 0.98 (*ADH2*·1) and 0.42 (*ADH3*·2). Participants with *ADH2* slow compared with participants with *ADH2* fast alcohol degradation were two to three times more likely to drink alcohol, and among alcohol drinkers, they had an approximately 30% higher alcohol intake (data not shown). Also, they were more often daily, heavy and excessive drinkers (Figure 3A, B, and C).

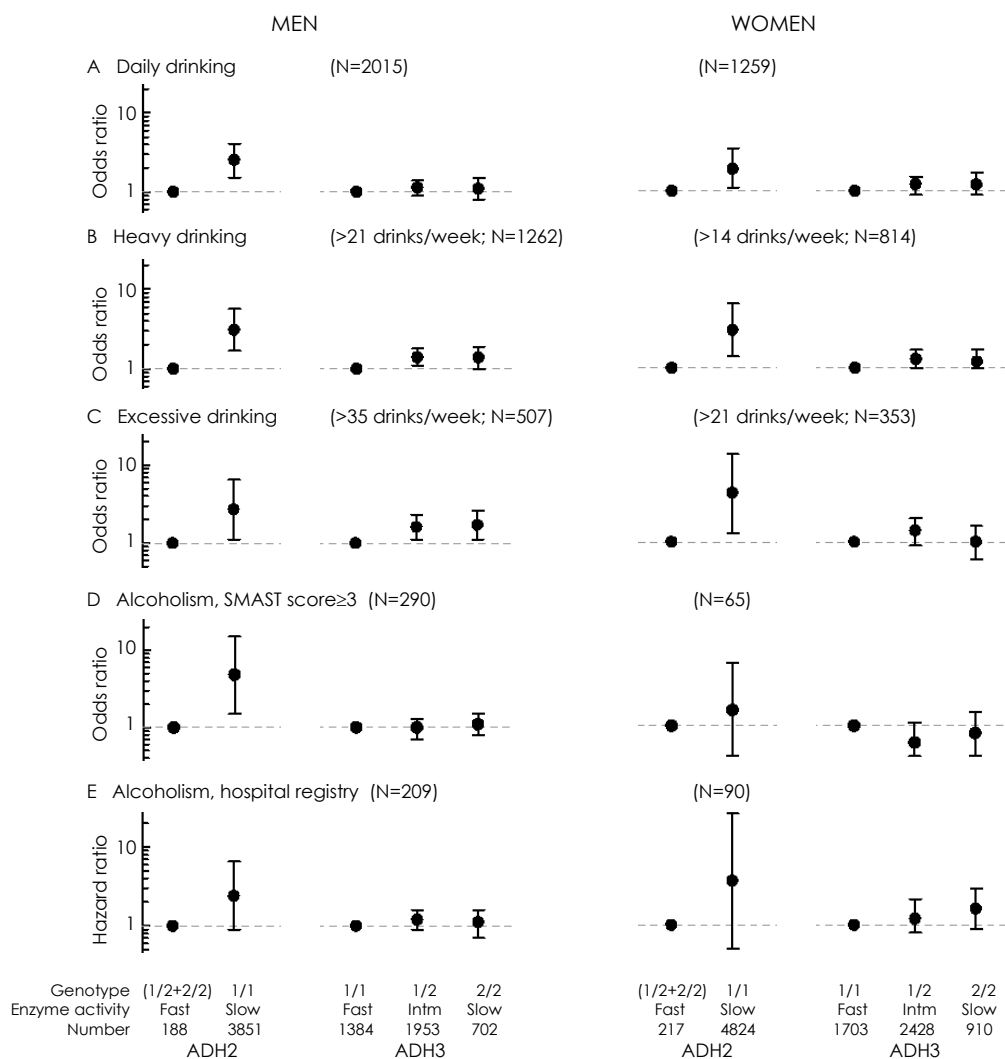


FIGURE 3. Sex-specific odds ratios for daily, heavy and excessive drinking and alcoholism (SMAST score  $\geq 3$ ) and hazard ratios for alcoholism (hospital registry information) by *ADH2* and *ADH3* genotypes. Number of cases in the respective analyses is noted beside each endpoint. Relative enzyme activity and total number of participants is noted at the bottom lines. Reference categories were *ADH2*·2/2+1/2 and *ADH3*·1/1. Estimates were adjusted for age, examination year, school education and other genotype.

Furthermore, there was a tendency that participants with *ADH2* slow versus fast alcohol degradation had a higher risk of alcoholism, as estimated from the *Short Michigan Alcoholism Screening Test* (SMAST) and hospital registry information (Figure 3D and E).

For *ADH3*, odds for heavy and excessive drinking were 40% to 70% higher among men who were heterozygous or homozygous for the slow alcohol degrading *ADH3*·2 allele than among men who were homozygous for the fast alcohol degrading *ADH3*·1 allele (Figure 3B and C). Similar results were found among women; however, effect sizes were slightly smaller and only statistically significant for heavy drinking.

Alleles of *ADH2* and *ADH3* are differently distributed in various ethnic groups. Frequencies of *ADH2*·1 and *ADH3*·2, coding for slow alcohol degradation are approximately 98% and 40% among Caucasians and only 30% and 10% among East Asians.<sup>54</sup> Hence, the genotype frequency of *ADH2*·1/1 is 95% among Caucasians and 9% among East Asians (Figure 4A). Odds ratios of heavy drinking and alcoholism according to *ADH2*·1/1 in the two populations are comparable (Figure 4B and C), but due to the different genotype distributions in the two populations, population attributable risks for heavy drinking and alcoholism are much higher among Caucasians than among East Asians. Therefore, population risks of heavy drinking and alcoholism attributable to the *ADH2*·1/1 genotype was 67% and 70% among Caucasians compared with 9% and 24% among East Asians (Figure 4D and E).

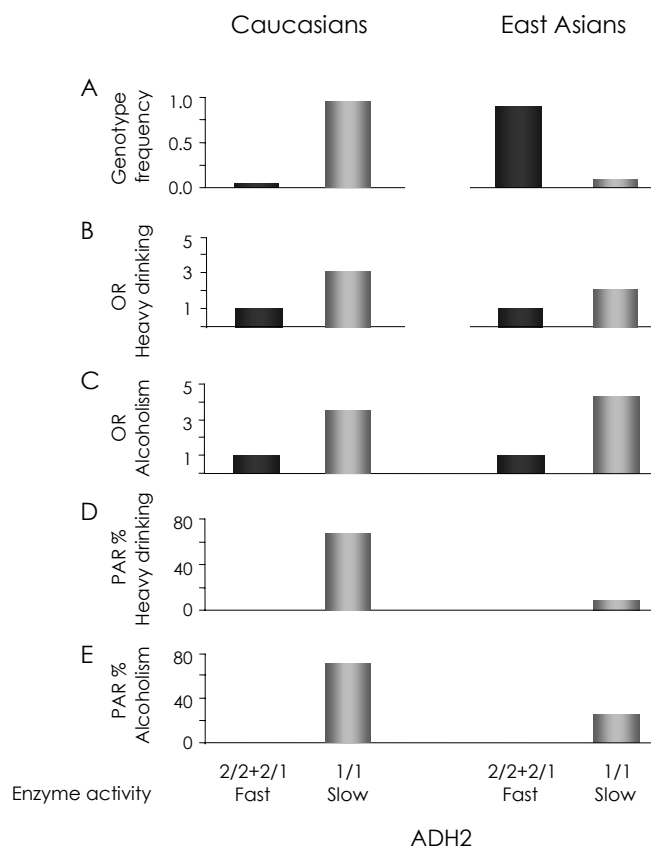


FIGURE 4. Genotype frequencies, odds ratios and population attributable risks of heavy drinking and alcoholism according to *ADH2* genotypes among Caucasians and East Asians (men and women combined). For East Asians, genotype frequencies and odds ratios were calculated from previous studies.<sup>54,55</sup> Reference category was *ADH2*·2/2+1/2. OR; odds ratio, PAR; population attributable risk.

## Study 2: Alcohol drinking pattern and coronary heart disease

During 302,857 person-years of follow up, 1283 men and 749 women from the Diet, Cancer and Health Study developed coronary heart disease. Among men, drinking frequency was inversely associated with risk of coronary heart disease over the whole range of drinking frequencies and the lowest risk was observed among daily drinkers (hazard ratio 0.59, 95% confidence interval 0.48 to 0.71, compared with men drinking on less than 1 day/week). Compared with women who drank alcohol on less than 1 day/week, women who drank alcohol on 1 day/week had a lower risk of coronary heart disease (0.64, 0.51 to 0.81); however, there was little difference between women who drank alcohol on 1 day/week, 2 to 4 days/week (0.63, 0.52 to 0.77), 5 or 6 days/week (0.79, 0.61 to 1.03), and 7 days/week (0.65, 0.51 to 0.84).

Exploring associations between drinking frequency and coronary heart disease within strata of amount of alcohol intake, inverse associations were consistently observed among men (Figure 5A), but not among women (Figure 5B). In contrast, exploring associations between amount of alcohol intake within strata of drinking frequency, inverse associations were consistently observed among women (Figure 5D), but not among men (Figure 5C).

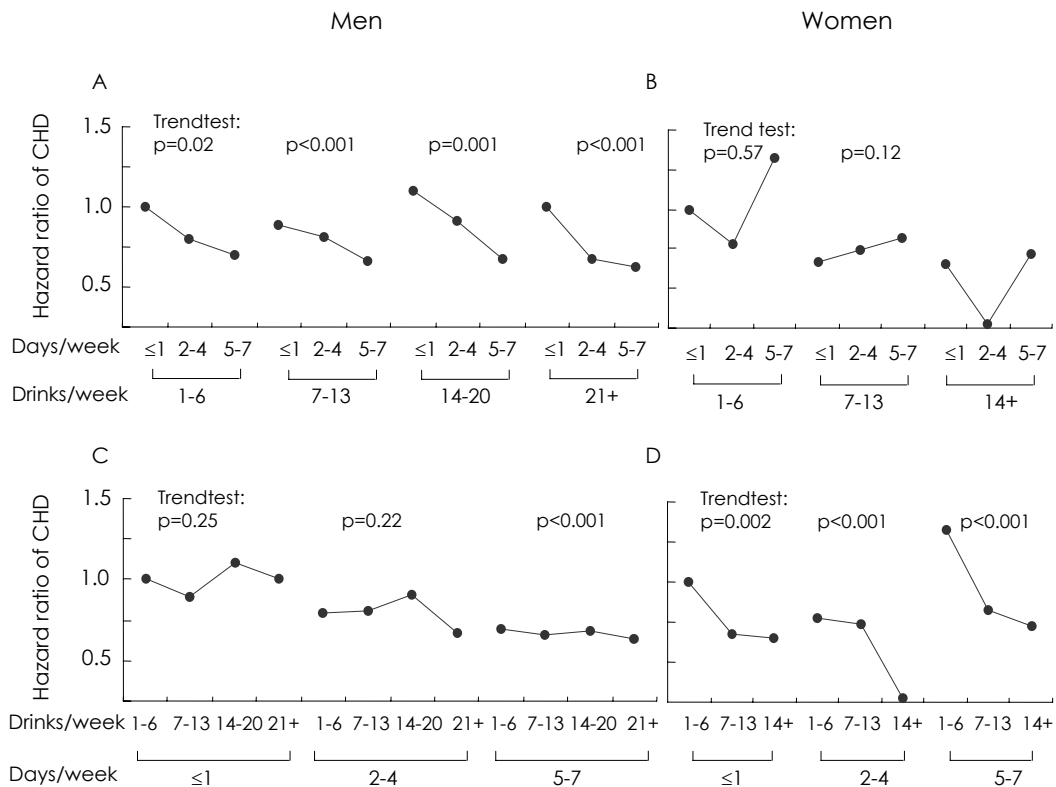


FIGURE 5. Sex-specific hazard ratios of coronary heart disease according to combinations of drinking frequency and amount of alcohol intake. Hazard ratios were adjusted for age, education, smoking, physical activity, body mass index, and intake of vegetables, fruit, fish and saturated fat. Participants drinking 1-6 drinks/week on ≤1 day/week were reference.

### Study 3: Alcohol drinking pattern and obesity

Among 25,325 men and 24,552 women from the Diet, Cancer and Health Study who were eligible for this study, 15% of men and 12% of women were obese (body mass index  $\geq 30$  kg/m<sup>2</sup>), 25% of men and 25% of women had large waist circumference ( $\geq 102$  centimetres, men;  $\geq 88$  centimetres, women) and 47% of men and 45% of women had small hip circumference ( $< 100$  centimetres).

Drinking frequency was inversely associated with obesity (Figure 6A and B) and with large waist circumference (Figure 6C and D), meaning that the most frequent drinkers had the lowest probability of being obese and the lowest probability of having large waist. A high drinking frequency was associated with small hip circumference (Figure 6E and F). Results were similar for men and women, and were consistent within strata of the weekly amount of alcohol intake (data not shown).

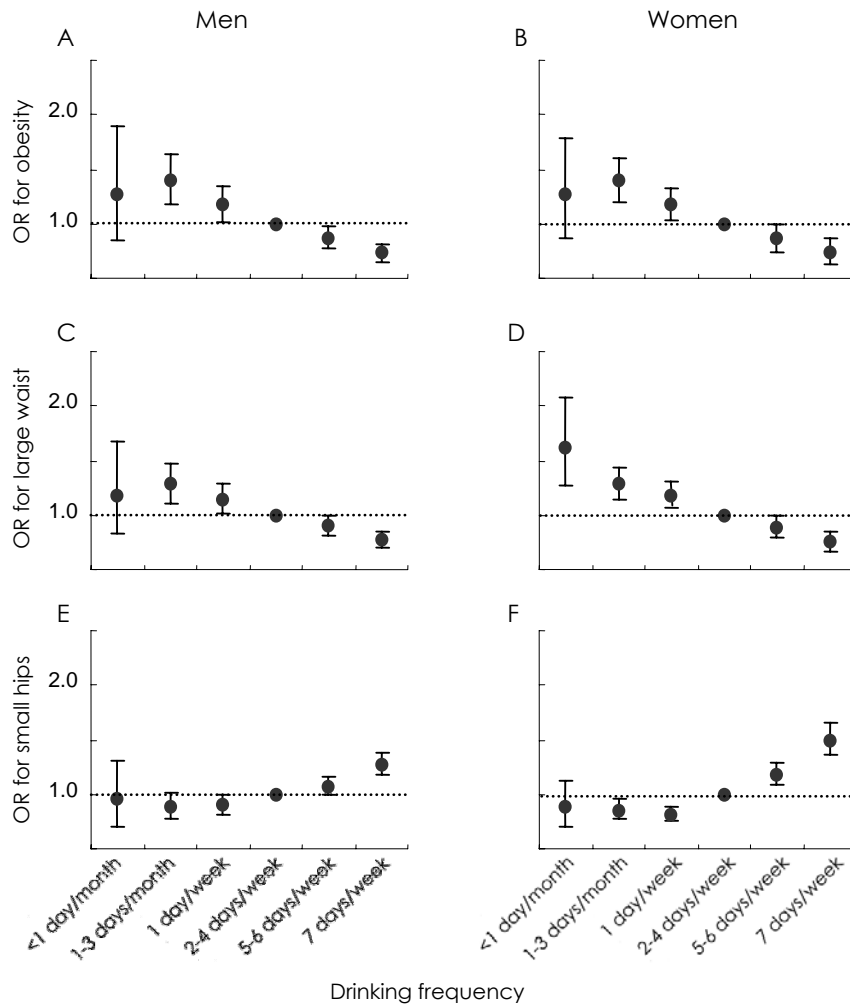


FIGURE 6. Sex-specific odds ratios for obesity, large waist and small hips according to alcohol drinking frequency. Odds ratios were adjusted for age, amount of alcohol intake, education, smoking, physical activity, and diet. Odds ratios for large waist and for small hips were also adjusted for BMI residuals. Obesity was defined as BMI  $\geq 30$  kg/m<sup>2</sup>, large waist was defined as  $\geq 102$  centimeters for men and  $\geq 88$  centimeters for women, and small hip circumference was defined as  $< 100$  centimeters. Reference category was participants drinking on 2 to 4 days/week. OR; odds ratio, BMI; body mass index.

#### Study 4: Alcohol drinking pattern and all-cause mortality

During 386,638 person-years of follow up in the Diet, Cancer and Health Study, 1528 men and 915 women died. Among both men and women, the well-known J-shaped curve between amount of alcohol intake and risk of all-cause mortality was observed (Figure 7). Furthermore, among men drinking more than 14 drinks/week and among women drinking more than 7 drinks/week, non-frequent drinkers had a higher risk of mortality than frequent drinkers (Figure 7). Hazard ratios also tended to increase with amount of alcohol intake among frequent drinkers. An overall test comparing frequent and non-frequent drinkers with a weekly intake of more than 1 drink/week was statistical significant (men:  $p=0.03$ , women:  $p=0.05$ ).

Exploring combinations of amount and frequency of alcohol intake in more detail, men drinking on 5 to 6 days/week and a weekly amount of 7 to 13 drinks or 14 to 21 drinks had the lowest hazard ratios (0.51, 95% confidence interval 0.36 to 0.73; and 0.52, 0.35 to 0.76), compared with men drinking less than 1 drink/week. For women, the lowest, although not statistical significant, hazard ratios were for drinking on 5 to 6 days/week and a weekly amount of 1 to 6 drinks (0.72, 0.32 to 1.64) and for drinking on 5 to 6 days/week and a weekly amount of 7 to 13 drinks/week (0.84, 0.56 to 1.27).

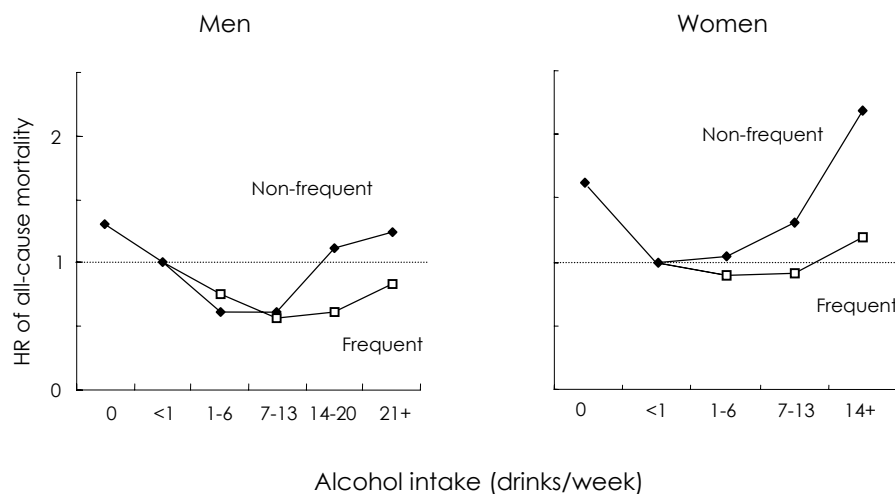


FIGURE 7. Sex-specific hazard ratios (HR) of all-cause mortality according to amount and frequency of alcohol intake. Non-frequent drinking was defined as drinking on less than two days/week and frequent drinking was defined as drinking on at least two days/week. Hazard ratios were adjusted for education, smoking, body mass index, physical activity, diet, and diseases before baseline. Reference category was participants drinking more than 0 and less than 1 drink/week ('<1').

## DISCUSSION

Can drinking patterns and alcoholism be predicted from genetic variation in *ADH2* and *ADH3*?

In our study of 9080 Caucasians, participants with *ADH2* slow versus fast alcohol degradation had a higher alcohol intake, were more often daily, heavy and excessive drinkers and had higher risk of alcoholism. Furthermore, individuals with *ADH3* slow versus fast alcohol degradation were more often heavy and excessive drinkers. In agreement with these results, a previous study in 334 Australian Caucasians found that *ADH2* slow versus fast alcohol degradation was associated with a higher alcohol intake in men, but not in women; for *ADH3*, no differences were found.<sup>56</sup> However, the Australian study had much less statistical power than ours.

Among men, we found relative estimates for alcoholism ranging from 2.1 to 4.8 among *ADH2*·1 homozygotes, which is comparable with results among East Asians (meta-analysis pooled odds ratio 4.3, 95% confidence interval 2.9 to 6.5).<sup>54</sup> Previous studies among Caucasians have found more modest effect sizes of alcoholism, however, the majority of these studies were underpowered and were not adjusted for sex and age (Table 1). Previous studies among Caucasians of *ADH3*, in agreement with our results, have not found associations between *ADH3* polymorphism and alcoholism (Table 1).

TABLE 1. Meta-analysis of previous studies of *ADH2* and *ADH3* genotype and alcoholism in Caucasians

	Country	Study design	Cases/control	Odds ratio (95% confidence interval)		
				<i>ADH2</i> ·1/1*	<i>ADH3</i> ·1/2†	<i>ADH3</i> ·2/2†
Vidal 2004 <sup>15</sup>	Spain	Case-control	♂ 264/255	0.9 (0.5-1.6)	0.8 (0.6-1.3)	0.9 (0.6-1.6)
Ogurtsov 2001 <sup>57</sup>	Russia	Case-control	♂♀ 110/50	2.4 (1.1-5.3)	NA	NA
Rodrigo 1999 <sup>58</sup>	Spain	Case-control	♂ 150/280	1.2 (0.6-2.4)	NA	NA
Grove 1998 <sup>59</sup>	England	Case-control	♂♀ 264/121	NA	1.0 (0.6-1.6)	1.8 (0.9-3.7)
Whitfield 1998 <sup>56</sup>	Australia	General population study	♂ 37/119 ♀ 27/151	6.4 (0.9-274) 0.4 (0.1-2.5)	NA	NA
Espinos 1997 <sup>60</sup>	Spain	Case-control	♂♀ 71/71	1.5 (0.6-4.4)	0.4 (0.2-1.0)	0.6 (0.2-1.7)
Sherman 1994 <sup>61</sup>	England	Case-control	♂♀ 26/16	NA	3.8 (0.5-48)	15 (1.0-∞)
Vidal 1993 <sup>62</sup>	Spain	Case-control	♂♀ 107/115	1.2 (0.6-2.6)	NA	NA
Gilder 1993 <sup>63</sup>	England	Case-control	♂♀ 82/84	1.2 (0.3-4.4)	1.0 (0.5-2.1)	1.5 (0.6-4.0)
Poupon 1992 <sup>64</sup>	France	Case-control	♂♀ 81/60	NA	0.8 (0.4-1.8)	0.5 (0.1-1.7)
Day 1991 <sup>65</sup>	England	Case-control	♂♀ 72/79	NA	0.6 (0.3-1.4)	0.6 (0.2-1.5)
Couzigou 1990 <sup>66</sup>	France	Case-control	♂♀ 46/39	0.6 (0.0-12)	NA	NA
Previous studies			♂♀ 894/1164 ♂♀ 858/686	1.2 (0.9-1.7)	0.8 (0.6-1.1)	1.0 (0.7-1.5)
Previous studies+ our study			♂♀ 1249/9889 ♂♀ 1213/9411	1.4 (1.1-1.9)	0.9 (0.7-1.1)	1.1 (0.8-1.3)

Odds ratios for each specific study were calculated using exact logistic regression on the basis of presented results in the original papers. Pooled odds ratios were calculated by logistic regression with a random effect for study. NA indicates that data were not available. Four studies reporting genotype frequencies not in Hardy-Weinberg equilibrium were omitted.

\*With *ADH2*·1/2+2/2 as reference. †With *ADH3*·1/1 as reference.

A likely explanation of our findings is that differences in enzyme activity from the *ADH2* and *ADH3* polymorphisms result in intra-individual differences in alcohol degradation and that, for a given level of alcohol intake, individuals with fast alcohol degradation have higher levels of acetaldehyde and thus more unpleasant symptoms compared with individuals with slow alcohol degradation. Thus, with slow alcohol degradation, individuals can enjoy the pleasure of high blood alcohol levels without the uncomfortable symptoms seen with high acetaldehyde levels.

Our results also suggest that *ADH2* and *ADH3* genotypes may partly explain why Caucasians generally drink more alcohol than East Asians. The population risk of heavy drinking and alcoholism attributable to the *ADH2*\*1/\*1 genotype was 67% and 70% among Caucasians in the present study, and only 9% and 24% among East Asians

In conclusion, the magnitude and consistency of observed associations and the biological plausibility adds to the evidence that *ADH2* and *ADH3* polymorphisms are causally related to alcohol drinking patterns and alcoholism.

Drinking pattern and coronary heart disease, evidence for sex-specific associations?

Our data suggest that drinking frequency is inversely and independently associated with risk of coronary heart disease among men. In contrast, results among women suggest that the weekly amount of alcohol intake is more important than drinking frequency for the inverse association with coronary heart disease.

Previous studies have addressed associations between drinking pattern and coronary heart disease (Table 2). A study with comparable measures of drinking pattern as our study also emphasizes frequency as the primary determinant among men.<sup>10</sup> Among women, a recent case-control study found inverse associations with both amount and drinking frequency, although there was a tendency that amount was more strongly associated than frequency.<sup>67</sup> Most studies with a measure of binge drinking (often defined as drinking more than a certain number of drinks per session as for instance eight)<sup>20</sup>, found increased risk among both men<sup>5,7,20</sup> and women<sup>8,20</sup> (Table 2).

Several explanations may account for sex specific differences in the association between drinking pattern and coronary heart disease. One explanation is sex-specific drinking habits, such as drinking with meals, which may contribute to a greater risk reduction than drinking outside meals.<sup>5</sup> It is possible that frequently drinking men are more likely to drink with meals than frequently drinking women. However, a favourable effect of meal-related alcohol intake is not found in all populations.<sup>10</sup> Another explanation could be a greater degree of residual confounding among women than among men. Interestingly, biomarkers suggested to explain the association between alcohol and decreased risk of coronary heart disease, such as high-density lipoprotein and fibrinogen, were found to explain a larger proportion of the association among men than among women, suggesting that alcohol has particular effects on mediators according to sex.<sup>67</sup> Other biological explanations for sex-specific associations include differences in alcohol pharmacokinetics and effects of alcohol on sex hormones. Some results suggest that men have more efficient first-pass metabolism for alcohol in the liver, while women may be eliminating alcohol faster than men.<sup>68</sup> Further, alcohol drinking is thought to increase oestrogen levels,<sup>69,70</sup> and endogenous oestrogen may have beneficial effects on the cardiovascular system, protecting women from coronary heart disease until menopause,

whereupon the incidence approaches the incidence among men.<sup>71</sup> It remains to be proven if any of these putative mechanisms depends upon the drinking pattern. Few women in this study were pre-menopausal and our findings may be limited to postmenopausal women.

In summary, our results suggest that there may be sex-specific associations between drinking frequency and coronary heart disease. These findings could be due to unobserved sex-specific drinking habits or to sex-specific associations between drinking frequency and cardiovascular mediators.



TABLE 2. Previous studies of drinking pattern and coronary heart disease

Author	Country	Study design	Population N (N <sub>Endpoints</sub> )	Measure of drinking pattern	Findings
Mäkelä 2006 <sup>4</sup>	Finland	Cohort	♂ 3481 (561) ♀ 2913 (263)	Amount divided into amount consumed on heavy and non-heavy drinking sessions.*	Inverse association for amount consumed on non-heavy drinking sessions and no association for amount consumed on heavy drinking sessions.
Mukamal 2005 <sup>67</sup>	USA	Nested case-control	♂ 798 (266) ♀ 747 (249)	Frequency Drinking with meals	Drinking 3-4 or 5-7 days/week implied lower risk than drinking <1 or 1-2 days/week (♂ and ♀).
Murray 2005 <sup>72†</sup>	Canada	Cohort	♂ 2526 (376)	Binge drinking (≥8 drinks per session).	No association
Trevisan 2004 <sup>5</sup>	Italy	Case-control	♂ 1332 (427)	Drinking outside meals Weekend drinking	Drinking outside meals and only in weekends implied higher risks compared with drinking mainly with meals or during the week [OR=1.5 (1.0-2.3) and 1.9 (1.2-3.0), respectively].
Mukamal 2003 <sup>10</sup>	USA	Cohort	♂ 38077 (1418)	Frequency and weekly amount combined. Drinking with meals.	Inverse association with frequency, and frequency seemed to be stronger associated than amount. Compared with drinking <1 day/week the RR for drinking 5-7 days/week was 0.6 (0.5-0.8). Findings were independent of the proportion of alcohol consumed with meals.
Laatikainen 2003 <sup>6‡</sup>	Finland	Cohort	♂ 5092 (123)	Binge drinking (≥6 drinks per session)	Binge drinking increase risk [HR 1.8 (1.0-3.1)].
Murray 2002 <sup>20</sup>	USA	Cohort	♂ 580 (59) ♀ 574 (28)	Binge drinking (≥8 drinks per session)	Binge drinking increase risk [♂: HR 2.3 (1.2-4.2), ♀: HR 1.1 (1.0-1.2)]
Malyutina 2002 <sup>7‡</sup>	Russia	Cohort	♂ 6502 (384)	Binge drinking (≥13 drinks per session compared with <7 drinks per session). Frequency and amount per session combined.	Binge drinking implies increased risk [HR 1.3 (0.8-2.0)]. Increased risk among frequent heavy drinkers. HR for drinking ≥3 days/week and ≥10 drinks/session was 1.8 (0.9-3.7) compared with drinking <1/week and ≥10 drinks/session.
Hammar 1997 <sup>8</sup>	Sweden	Case-referent	♂ 1569 (289) ♀ 760 (140)	Binge drinking (1/2 a bottle of spirits or intoxication).	♂: No association. ♀: HR 1.8 (0.9-3.7) for binge drinking.
McElduff 1997 <sup>9</sup>	Australia	Case-control	♂ 9712 (6685) ♀ 5918 (2880)	Frequency and amount per session combined.	Both frequency and amount per session are associated. ♂: Lowest risk for drinking 5-6 days/week and 1-4 drinks/session. ♀: Lowest risk for drinking 5-6 days/week and 1-2 drinks/session.

\* Heavy drinking sessions defined as blood alcohol concentration ≥ 0.1% † Heavy drinkers excluded at study entry ‡ Endpoint was deaths from coronary heart disease. Cohort; general population cohort, OR; odds ratio, RR; relative risk, HR; hazard ratio.

Is drinking pattern independently associated with obesity?

We observed inverse associations between alcohol drinking frequency and odds ratios of obesity and large waist; frequently drinking participants were less likely to be obese and to have large waists than less frequently drinking participants. Associations were similar among men and women and were independent of the amount of alcohol intake. In agreement with these results, one other study has found that waist circumference was inversely associated with drinking frequency (reported as monthly, weekly and daily drinking).<sup>73</sup>

The most important limitation of this study is the cross-sectional design; information on alcohol and anthropometric measures were obtained at the same time. Hence, it is not possible to determine the causal relationship for the observed associations. It cannot be excluded that being obese may cause different alcohol drinking patterns than being lean. However, if the observed associations between drinking frequency and obesity represent a causal relation, a possible biological mechanism is differential induction of the *microsomal ethanol-oxidising system* by drinking frequency. While the bulk of ingested alcohol is degraded by alcohol dehydrogenase, microsomal ethanol-oxidising system is induced by heavy, regular alcohol intake.<sup>74</sup> It has been suggested that alcohol dehydrogenase and microsomal ethanol-oxidising system in conjunction constitute a futile cycle, so that energy from alcohol is resulting mostly in increased thermogenesis. If such a cycle is of any physiological significance, drinking frequency may be important for the degree of microsomal ethanol-oxidising system activation, and hence for the fraction of energy from alcohol that is lost as heat.<sup>75</sup> Another mechanism could be that low doses of alcohol stimulate energy expenditure because alcohol has an acute thermogenic effect.<sup>76</sup> It is possible that, for the same level of weekly alcohol intake, a frequent drinking pattern results in relatively more energy being converted to heat, compared with a less frequent intake.

In summary, we observed strong inverse associations between drinking frequency and obesity. If our results represent causal associations, obesity may explain part of the association between alcohol-drinking pattern and coronary heart disease, since obesity is a well-known risk factor for coronary heart disease.

Is the J-shaped all-cause mortality curve influenced by drinking pattern?

We found that drinking pattern influenced the J-shaped relation between alcohol intake and all-cause mortality. For the same amount of alcohol consumption, a non-frequent intake implied a higher risk of death than a frequent intake.

Previous studies have examined the association between drinking pattern and all-cause mortality (Table 3). Although other measures of drinking patterns are used, results consistently imply a hazardous effect of drinking large amounts of alcohol per session.<sup>4,6,36,77</sup> We did not have the ability to identify participants with a binge-like drinking pattern, except for non-frequent drinkers with a high weekly intake, who logically must drink several drinks per session. In our study, two types of participants, the first of whom drinks two drinks each day and the second who drinks one drink each day plus seven additional drinks on Saturday nights, report the same weekly amount and drinking frequency, representing two different drinking patterns the latter of which may be associated with a higher risk of mortality than the former. Hence, the observed risks

among frequent drinkers are a mixture of risks for frequent drinkers with a binge-like drinking pattern and frequent drinkers without a binge-like drinking pattern. If we were able to adjust for binge drinking, risks among the frequent drinkers would possibly be lowered.

In summary, we observed the well-known J-shaped curve between amount of alcohol intake and risk of all-cause mortality, but the risk was generally higher among participants with a non-frequent intake than among participants with a frequent intake.

Should public advice on sensible drinking include a message on drinking pattern?

For public health purposes, the association between alcohol and all-cause mortality is relevant because it reflects net loss of life attributable to alcohol consumption and thus constitutes the scientific basis for creating guidelines on sensible drinking. In 1990, the Danish National Board of Health introduced the *sensible drinking limits*, advising the public not to exceed a certain amount of alcohol intake per week (14 drinks/week for women and 21 drinks/week for men). In the light of our and previous results,<sup>32-35</sup> these guidelines seem reasonable; at these levels of alcohol consumption, the risk of mortality is not increased compared with non-drinkers, at least not among the frequent drinkers (Figure 7). Countries like for instance the United Kingdom have comparable guidelines for sensible amount of alcohol drinking, which since 1994 furthermore included advice on sensible drinking pattern, more precisely not to drink more than three and four drinks per session for women and men. In the autumn of 2005, the Danish guidelines for sensible drinking were expanded to also comprise drinking pattern, advising men and women not to drink more than five drinks on any session. Considering the emerging and consistent evidence that the beneficial effects of alcohol is not attained by episodic binge drinking, and that all-cause mortality is increased among individuals with a binge-like drinking pattern, this seem to be of considerable public health relevance. Another potential important factor however not further discussed in this thesis is age: among the young, the association between alcohol intake and all-cause mortality, and especially drinking pattern and all-cause mortality is sparsely examined. Our study population consisted of middle-aged men and women and results are thus conditional for having survived until 50 to 65 years. This age group is at high risk of coronary heart disease and qualifies for studying beneficial effects of alcohol. For younger individuals, the risk of coronary heart disease is low and beneficial effects of alcohol are probably negligible. Hence, the detrimental effects of alcohol, such as increased risk of traffic accidents and injuries most likely predominate. Therefore, the current guidelines for sensible drinking may not be sensible for the young.

TABLE 3. Previous studies of drinking pattern and all-cause mortality

Author	Country	Population N (N <sub>Endpoints</sub> )	Study design	Measure of drinking pattern	Findings
Mäkelä 2006 <sup>4</sup>	Finland	♂ 3481 (746) ♀ 2913 (398)	Cohort	Amount divided into amount consumed on heavy and non-heavy drinking sessions*	♂ Increased risk in the highest category of alcohol consumed on heavy drinking sessions, and no increased risk for amount of alcohol consumed in non-heavy drinking sessions. Highest drinking category for heavy and non-heavy drinking sessions was $\geq 7$ drinks/week. ♀ No association for alcohol consumed on heavy drinking sessions, and inverse association with amount of alcohol consumed in non-heavy drinking sessions. Highest drinking category for heavy and non-heavy drinking sessions was $\geq 1.5$ drinks/week.
Laatikainen 2003 <sup>6</sup>	Finland	♂ 5092 (347)	Cohort	Binge drinking ( $\geq 6$ drinks per session)	Binge drinking increases risk [HR=1.6 (1.2-2.1)].
Malyutina 2002 <sup>7</sup>	Russia	♂ 6502 (836)	Cohort	Binge drinking ( $\geq 13$ drinks per session compared with $< 7$ drinks per session). Frequency and amount per session combined.	No association for binge drinking. Increased risk among frequent heavy drinkers. HR for drinking $\geq 3$ days/week and $\geq 10$ drinks/session was 1.6 (1.0-2.5) compared with drinking $< 1$ /week and $\geq 10$ drinks/session.
Trevisan 2001 <sup>77</sup>	Italy	♂ 7688 (457) ♀ 4326 (107)	Cohort	Drinking outside meals	Drinking outside meals implied higher risk compared with drinking mainly with meals [♂ OR 1.5 (1.1-2.0) and ♀ 5.0 (1.5-11), respectively].
Rehm 2001 <sup>36</sup>	USA	♂ 2037 (272) ♀ 3035 (260)	Cohort	Binge drinking ( $\geq 8$ drinks per session at least monthly or intoxication)	♂ Binge drinking implies increased risk [HR 1.6 (0.9-3.1)] ♀ No association.

\* Heavy drinking sessions defined as blood alcohol concentration  $\geq 0.1$  %  
Cohort; general population cohort, OR; odds ratio, HR; hazard ratio.

## Sources of bias - alternative explanations for obtained results?

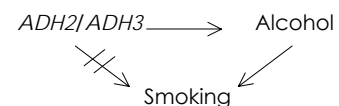
### *Confounding*

An important potential confounder in a genetic association study is ethnic stratification, i.e. that genotype distribution varies across subgroups of the population and at the same time, baseline risk differs according to these subgroups.<sup>78</sup> Distributions of *ADH2* and *ADH3* genotypes show marked variation across ethnic groups,<sup>12</sup> and ethnicity is likely associated with alcohol drinking patterns and risk of alcoholism. Therefore, participants born outside Denmark were excluded. This procedure has limitations; it was not possible to differ between individuals of Danish and of non-Danish ethnicity born abroad meaning that some participants of Danish ethnicity were excluded. This exclusion is, however, most likely nondifferential according to genotype, and leads only to a minor reduction in effective study size. Another limitation is that, by this procedure, it was not possible to exclude participants of non-Danish ethnicity born in Denmark. However, considering the facts that genotypes were in Hardy-Weinberg equilibrium, and that the *ADH2*:2 frequency was comparable with findings in other Caucasian populations,<sup>12</sup> speak against a major residual confounding from ethnic stratification.

An alternative explanation of our results on *ADH* genotypes is that untyped variants are in linkage disequilibrium with the *ADH2* and *ADH3* polymorphisms, meaning that our results stem from indirect associations. In that case, the causal variant would be localised within the *ADH2* and/or *ADH3* loci or within nearby genes (or regions regulating gene transcription), most likely within other genes in the *ADH* gene cluster (Figure 1). If so, the function of unidentified causal variants would probably alter alcohol degradation rate and results would still represent the same biological mechanism. Unless the typed *ADH2* and *ADH3* polymorphisms were perfect surrogates for the causal variants, obtained results would underestimate the direct association.

Estimates for *ADH2* and *ADH3* genotypes were not adjusted for smoking or other modifiable factors. Smoking and alcohol are associated lifestyle behaviours, but it is unlikely that *ADH2* and *ADH3* genes are associated with smoking through other pathways than alcohol (Figure 8). In this case, an association between genes and smoking would result from an effect of genes on alcohol, and smoking would thus not have the properties of a confounding factor.<sup>79</sup> Ultimately, adjustment for smoking could lead to attenuated estimates.

FIGURE 8. Alcohol and smoking are associated behaviours, but because any association between *ADH* genes and smoking is likely to result of an effect of genes on alcohol, smoking is not a confounder.



Residual confounding or confounding from uncontrolled risk factors could have caused bias in the observed associations between drinking pattern and health endpoints (Studies 2, 3, and 4). Suggestions of such factors include social factors since high volumes of alcohol per drinking session is shown to be associated with negative social circumstances.<sup>80</sup> School education was used as a proxy for social status, but more detailed

information on other social factors would have been desirable. For instance, marital status, social network and socio-economic position are likely linked with both drinking patterns and health status, and could hence be confounding results.<sup>81,82</sup>

Adjustment had minor influence on results for drinking pattern and coronary heart disease. For example, adjustment for potential confounders changed hazard ratios from 0.65 to 0.71 among men drinking 5 to 6 days/week, and from 0.60 to 0.59 among daily drinking men. Most influence of adjustment was that due to adjustment by smoking. Residual confounding is possible; dimensions such as smoking duration and passive smoking were not accounted for. However, this would most likely have attenuated results, because smoking and alcohol are positively associated behaviours and smoking are positively associated with risk of coronary heart disease. It is not likely that wine drinking, which may be more beneficial than beer and spirits,<sup>18</sup> are causing our results, because it has previously been shown that wine drinkers in this cohort actually drink less frequently than beer drinkers.<sup>83</sup> Thus, confounding from wine would most likely have attenuated results. In analyses of drinking frequency and obesity, adjustment also had limited influence.

In analyses of drinking pattern and all-cause mortality, adjustment for potential confounders (smoking, physical activity, body mass index, diet, school education and diseases) generally reduced the difference between frequent and non-frequent drinkers and hence the importance of drinking pattern (please refer to Figure 1 in Paper 4).

In conclusion, there is no reason to suspect that obtained results from the study of *ADH* genes is greatly influenced by confounding. Results from studies of drinking pattern, coronary heart disease, obesity and mortality may be confounded by social factors.

#### *Misclassification of exposures*

Misclassification of *ADH2* and *ADH3* genotypes could arise from preanalytical sources such as contamination by foreign DNA. To estimate the extent of such errors, DNA from new blood samples must be extracted and analysed, meaning that participants should be contacted and invited for reexamination. Due to the costs of such a validation, this was not feasible. Errors may arise during analyses because of contamination from one sample to another or because of incorrect interpretation of the signal from the chip. The latter error is more likely if the magnitude of the signal is relatively low compared to the background signal. Therefore, to minimise such errors, a relatively low value of the background signal was tolerated, and in the case of ambiguous signals, samples were rerun. Also, samples of known genotype were included on each chip to control the quality of each assay. Post analytical errors could arise due to incorrect registration into the database. To avoid this error, two independent laboratory technicians independently checked all results and database entries. Finally, mistaken identity of samples could occur through either of the analytical phases.

Any of the above mentioned errors in the assessment of *ADH2* and *ADH3* genotypes are probably nondifferential according to endpoint, and are thus unlikely to explain the obtained results. Furthermore, the observed genotype distribution complies with the expected distribution as predicted from the law of Hardy-Weinberg: in a population of random mating individuals with no selection pressure for either of the genotypes, the

distribution of genotypes follows this law. If not, genotyping errors are often responsible.

Drinking frequency and amount of alcohol intake could also be misclassified, which could lead to significant bias if the misclassification is differential. For instance, it is likely that participants have cut down on alcohol in response to early symptoms of coronary heart disease (so-called *sick quitters*<sup>84</sup>), causing a falsely high incidence rate among participants in the low alcohol categories and resulting in an overall inverse association. In order to evaluate the influence of sick quitters, sensitivity analyses were performed where early cases were excluded. This did not change results, which argues against that the inverse association between drinking frequency and coronary heart disease is explained by this potential bias.

In conclusion, we do not have reason to believe that the obtained results are considerably affected by misclassification of any of the exposures.

#### *Misclassification of endpoints*

The various endpoints are probably subject to some misclassification, i.e. sensitivity and/or specificity less than 100 percent. For the study on *ADH2* and *ADH3* genotypes, measures of heavy and excessive drinking will be misclassified if amount of alcohol intake is under- or overreported. This error is likely independent of genotype, and because endpoints are binary, will lead to bias towards the null.<sup>79</sup> The same applies for error in the definition of alcoholism by questionnaire (SMAST score). For alcoholism defined by hospital registry information, sensitivity is likely considerably less than 100 percent because many alcoholics are untreated or treated at private clinics not registered in the national registers. However, specificity could be close to perfect; few non-alcoholics are presumably diagnosed as alcoholics. In this scenario, non-differential misclassification is not affecting the hazard ratio.<sup>85</sup>

Misclassification of the coronary heart disease diagnosis occurs if patients fulfilling criteria for coronary heart disease were not diagnosed (that is, sensitivity less than 100 percent) or patients not fulfilling criteria for coronary heart disease were diagnosed as such (that is, a non-zero false positive rate). In order to explain our results, misclassification will have to be differential (i.e. to depend on drinking frequency); for example, a relatively lower sensitivity among frequent drinkers would cause an apparent inverse association between drinking frequency and coronary heart disease. This could occur if for instance frequent drinkers, who were more often smokers, were more likely to be misdiagnosed with lung diseases instead of correctly being diagnosed with heart disease.

The influence on hazard ratios of various scenarios of misclassifications of coronary heart disease is shown in Table 4.<sup>53</sup> In order to explain the decreased hazard ratio in daily drinking men, the nondifferential misclassification must be substantial. For example, assuming perfect sensitivity in the reference category and 60 percent among daily drinkers, and a false positive rate of zero in both groups, the true hazard ratio would be 1.0. Sensitivity analyses with similar scenarios were performed for other categories of drinking frequency and for women. Hazard ratios were generally robust unless a high degree of differential misclassification was assumed. Therefore, misclassification of disease status is unlikely to considerably have affected our results.

TABLE 4. Hazard ratios among men drinking 7 days/week compared with men drinking less than 1 day/week corrected for various scenarios of differential and nondifferential misclassification of the coronary heart disease diagnosis. Diagonal cells (underlined) represent scenarios with nondifferential misclassification.

<1 day/week (reference)		7 days/week						
Sensitivity	FP rate	Sensitivity	1	0.8	0.8	0.8	0.6	0.6
		FP rate	0	0	2	4	0	2
1	0		<u>0.60</u>	0.75	0.56	0.37	1.00	0.74
0.8	0		0.48	<u>0.60</u>	0.45	0.29	0.80	0.60
0.8	2		0.56	0.71	<u>0.53</u>	0.35	0.94	0.70
0.8	4		0.69	0.86	0.64	<u>0.42</u>	1.15	0.86
0.6	0		0.36	0.45	0.33	0.22	<u>0.60</u>	0.45
0.6	2		0.42	0.53	0.39	0.26	0.71	<u>0.53</u>
0.6	4		0.52	0.65	0.48	0.32	0.86	<u>0.42</u>

Note: Hazard ratios were calculated as incidence rate ratios. FP rate; false positive rate (events per 1000 person years; overall incidence rate among men was 9.2 per 1000 person years).

In the study of drinking pattern and obesity, endpoint measures (body mass index, waist and hip circumference), were obtained at the study clinic by trained personnel. Errors can be due to person-to-person variations in measurement method or incorret data entry. These errors are in all probability nondifferential and not likely to explain the observed associations.

For all-cause mortality, sensitivity and false positive rate were in all probability close to perfect and zero, respectively. Misclassification could be due to incorrect or lack of data entry into the Civil Registration System and is most likely nondifferential. Specificity is probably close to perfect and in that case, the non-differential misclassification is not affecting the hazard ratio.<sup>85</sup>

In conclusion, we have no reason to believe that the obtained results are considerably affected by misclassification of any of the studied endpoints.

#### *Selection bias*

Selection bias can occur as a consequence of nonparticipation. Individuals, who choose not to participate in the Copenhagen City Heart Study had higher all-cause mortality, and higher incidence of alcoholism and alcoholic liver cirrhosis than individuals who participated (Figure 9). This indicates that participants were at better health than nonparticipants, and that alcoholism and heavy alcohol drinking are underestimated compared with the underlying population. In other words, alcohol drinking patterns and alcoholism were associated with nonparticipation (endpoints in Study 1). If genotypes were also associated with nonparticipation, this could have lead to selection bias.<sup>86</sup> However, as none of the invited persons were aware of their genotype, this is not likely, and associations between *ADH* genotypes and alcohol endpoints are unlikely to be affected by selection bias.<sup>87</sup>



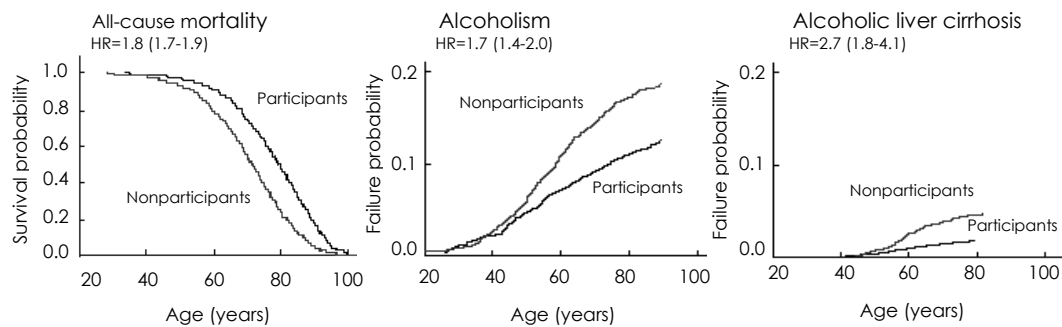


FIGURE 9. All-cause mortality, alcoholism and alcoholic liver cirrhosis among participants and nonparticipants of the Copenhagen City Heart Study 1991-1994. HR: Hazard ratios for non-participants compared with participants, adjusted for sex.

In the Diet, Cancer and Health Study, participation rate was 35 percent. Data to perform analyses on nonparticipants were not available, but a similar tendency as in the Copenhagen City Heart Study is likely. If so, heavy alcohol drinking and poor health were both causes of nonparticipation, and individuals with both causes are likely to be underrepresented. In other words, heavy drinkers who participate may be at better health than heavy drinkers in the population, whereas light and moderate drinkers who volunteer may be more representative of moderate and nondrinkers in the population. Ultimately, this may result in an apparent inverse association between alcohol and endpoint, even if there is no such association in the underlying population.<sup>86</sup> We observed inverse associations between drinking frequency and obesity and coronary heart disease and direct associations between alcohol intake and all-cause mortality. Hence, assuming selection bias were present as outlined above, results on mortality would probably have been biased towards the null, whereas results on coronary heart disease and obesity would have been biased away from the null. There is no definitive test for evaluating the presence or magnitude of this bias, but the facts that adjustment for diseases at baseline did not have major effects, that the observed number of coronary heart disease cases in the cohort during followup did not differ from the expected, that expected patterns for other risk factors such as smoking, body mass index and school education was observed, and that all-cause mortality as expected was increased among the most heavy drinking individuals, speaks against our results being caused by selection bias.<sup>88</sup>

In conclusion, selection bias is not likely to explain the obtained results in studies of *ADH* genotypes and all-cause mortality. It cannot be entirely excluded that selection bias may have had some impact on results in studies of coronary heart disease and obesity.

#### *Loss to follow-up*

In the Copenhagen City Heart Study and in the Diet, Cancer and Health Study, less than one percent of participants were lost during follow-up. Such minor loss cannot have had significant effect on any of our results.

## CONCLUSIONS AND PERSPECTIVES

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Findings from the study of genetic variation in genes coding for alcohol degrading enzymes suggest that this partly predicts the individual's drinking pattern and risk of alcoholism.

- Slow versus fast alcohol degradation is associated with a higher alcohol intake, with daily, heavy and excessive drinking and with risk of alcoholism.

Findings from studies on drinking pattern and coronary heart disease, obesity and all-cause mortality consistently point toward important and independent effects of drinking pattern.

- For coronary heart disease, drinking frequency may be the primary determinant of the inverse association between alcohol intake and coronary heart disease among men. For women, however, amount of alcohol may be more important than frequency.
- Drinking frequency and obesity was inversely associated, so that for a given amount of alcohol intake, the most frequent drinkers had the lowest probability of being obese and of having large waist circumferences. If this finding represent causal association, it is likely that obesity can explain part of the association between drinking frequency and coronary heart disease.
- For the same weekly amount of alcohol intake, a non-frequent intake implies a higher risk of all-cause mortality than a frequent one.

Examining various sources of bias (confounding, misclassifications and selection bias) gave no reason to believe that each single bias is likely to considerably have affected our results.

More needs to be learned about effects of alcohol dehydrogenase genes on specific measures of drinking patterns, as for instance binge drinking, and on context-dependent effects (gene-gene and gene-environment interactions).

The association between drinking pattern and coronary heart disease should be investigated in other studies, with emphasis on studying differences between men and women, including if alcohol pharmacokinetics and sex hormones can explain a potential sex-specific associations.

The association between drinking pattern and obesity should be examined in a prospective design with the possibility of deciding the temporality. Causal inference, followed by public health implications based on isolated findings like these should not be drawn. Given the rise of obesity prevalence in Western societies, a possible causal association between drinking pattern and obesity could be of considerable public health relevance.

Future cohorts with purposes of addressing alcohol exposure and health endpoints should include measures of drinking pattern, preferable characterising amount,

frequency and episodes of heavy drinking (binge drinking). Also, future studies should take social factors such as socio-economic status and social network into account.

The increased risk of mortality among non-frequent drinkers should be examined in more detail, with emphasis on studying causes of deaths. If, for instance the excess mortality mainly is due to external causes, such as traffic accidents, preventive strategies could with advantage focus on high-risk groups. Also, associations between drinking pattern and mortality should be examined in other age groups, especially in the young, among whom health effects of alcohol are predominantly detrimental, and hazardous implications of different drinking patterns may be considerably greater than among the middle-aged.

In the light of results of this thesis, it may seem that regular compared with episodic drinking is less health damaging. However, the 'optimal' drinking pattern is likely to depend on the nature of the endpoint: the risk of alcohol related conditions such as liver cirrhosis may be lower among episodic drinkers than among regular drinkers, because the liver is allowed to reconstitute between drinking sessions. Furthermore, habitual heavy alcohol drinking is expectedly associated with many adverse social circumstances and diseases, regardless of the drinking pattern.

## SUMMARY IN ENGLISH

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This thesis is based on studies conducted in the period from 2003 to 2006 at Center for Alcohol Research, the National Institute of Public Health in cooperation with the Department of Clinical Biochemistry, Herlev University Hospital. Obtained results are presented in four scientific papers three of which are published, and one is submitted.

Main purposes of the thesis were to examine genetic predictors of alcohol-drinking patterns and of alcoholism, and to examine associations between drinking pattern and coronary heart disease, obesity and all-cause mortality.

Alcohol is degraded in the liver by alcohol dehydrogenase (ADH), which is a class of isoenzymes. Genetic variation with functional implications exists in *ADH2* and *ADH3*, resulting in different alcohol degradation rates. By genotyping 9080 participants in the Copenhagen City Heart Study, we found that participants with *ADH2* slow versus fast alcohol degradation had an approximately 30 percent higher alcohol intake, more often drank alcohol every day and more often were heavy and excessive drinkers. Also, there was a tendency that participants with *ADH2* slow versus fast alcohol degradation had higher risks of alcoholism. For *ADH3*, we found that participants with slow versus fast degradation more often were heavy and excessive drinkers.

Among more than 50.000 participants in the Diet, Cancer and Health Study, we found an inverse association between drinking frequency and risk of coronary heart disease for men and this association seemed to be independent of the amount of alcohol intake. Among women, we found an inverse association between amount of alcohol and risk of coronary heart disease, which seemed to be independent of drinking frequency.

Within the same cohort, we found among both men and women an inverse association between drinking frequency and prevalence of obesity, so that participants, who were drinking alcohol frequently were less likely to be obese than participants, who drank less frequently. Results were similar for men and women and were independent of the amount of alcohol intake.

Also in the Diet, Cancer and Health Study, we compared mortality risk among participants with different drinking frequencies. We found that the risk was higher among women drinking 7 drinks/week and among men drinking 14 drinks/week if this amount was taken on one day of the week compared with distributing the same amount on more days of the week.

In conclusion, genetic variation in alcohol degrading enzymes is partly predicting drinking patterns and alcoholism. Drinking patterns is independently associated with risk of coronary heart disease (among men) and all-cause mortality, and with prevalence of obesity. These results are important for future studies of the biological effects of alcohol on health and for public guidelines on alcohol.

## SUMMARY IN DANISH

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Denne afhandling bygger på studier, som er gennemført i perioden 2003 til 2006 på Center for Alkoholforskning, Statens Institut for Folkesundhed i samarbejde med Klinisk Biokemisk Afdeling på Herlev Universitetshospital. Afhandlingen er baseret på fire videnskabelige artikler, hvoraf de tre er publiceret, mens en er indsendt.

Afhandlingens overordnede formål er at undersøge genetiske prædiktorer for alkohol-drikkemønstre og for alkoholisme, samt at undersøge drikkemønstrets betydning for koronar hjertesygdom, fedme og dødelighed.

Alkohol nedbrydes i leveren af alkoholdehydrogenase (ADH), som er en klasse af isoenzymer. Der findes genetisk variation i *ADH2* og *ADH3*, hvilket forårsager forskellig alkoholnedbrydningshastighed. Ved genotypebestemmelse af 9080 deltagere i Østerbroundersøgelsen fandt vi, at personer med *ADH2* langsom versus hurtig alkoholnedbrydning drak cirka 30 procent mere alkohol, oftere drak alkohol hver dag og oftere overskred Sundhedsstyrelsens genstandsgrænser (14 genstande om ugen for kvinder og 21 genstande om ugen for mænd). Der var desuden en tendens til at deltagere med *ADH2* langsom versus hurtig alkoholnedbrydning havde en højere risiko for alkoholisme. For *ADH3* fandt vi at deltagere med langsom versus hurtig alkoholnedbrydning oftere overskred Sundhedsstyrelsens genstandsgrænser.

Blandt de mere end 50.000 deltagere i Kost, Kræft og Helbreds kohorten fandt vi blandt mænd en invers sammenhæng mellem alkoholdrikkefrekvens og risiko for koronar hjertesygdom. Denne sammenhæng syntes at være uafhængig af det samlede ugentlige alkoholforbrug. Blandt kvinder fandt vi en invers sammenhæng mellem det ugentlige alkoholforbrug og risiko for koronar hjertesygdom, som syntes at være uafhængig af drikkefrekvensen.

I samme kohorte fandt vi blandt både mænd og kvinder en invers sammenhæng mellem drikkefrekvens og forekomst af fedme, således at deltagere, der drak alkohol hyppigt var slankere end deltagere, som drak mindre hyppigt. Denne sammenhæng syntes at være uafhængig af det samlede ugentlige alkoholforbrug.

Ligeledes i Kost, Kræft og Helbreds kohorten sammenlignede vi risikoen for at dø mellem deltagere med forskellig drikkefrekvens. Vi fandt at risikoen for at dø var større hvis et ugentligt forbrug på over 7 genstande for kvinder og over 14 genstande for mænd blev indtaget på en dag sammenlignet med at sprede et tilsvarende forbrug på flere af ugens dage.

Konkluderende kan siges, at genetisk variation i alkoholnedbrydende enzymer til en vis grad prædikerer drikkemønstre og alkoholisme. Alkohol-drikkemønstre har, uafhængigt af det samlede alkoholforbrug, indflydelse på risiko for koronar hjertesygdom (for mænd) og for død, og måske på fedmeudvikling. Disse resultater har betydning for såvel fremtidige studier af biologiske effekter af alkohols betydning for helbred som for folkesundhedsmæssige budskaber om alkohol.

## LIST OF CITED LITERATURE

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1. Rehn N, Room R, and Edwards G: Alcohol in the European region - consumption, harm and policies. World Health Organization 2001, [www.euro.who.int](http://www.euro.who.int)
2. Han C, McGue MK and Iacono WG: Lifetime tobacco, alcohol and other substance use in adolescent Minnesota twins: univariate and multivariate behavioral genetic analyses. *Addiction* 1999, 94; 981-993.
3. Kendler KS, Neale MC, Heath AC, Kessler RC and Eaves LJ: A twin-family study of alcoholism in women. *American Journal of Psychiatry* 1994, 151; 707-715.
4. Makela P, Paljarvi T and Poikolainen K: Heavy and nonheavy drinking occasions, all-cause and cardiovascular mortality and hospitalizations: a follow-up study in a population with a low consumption level. *Journal of Studies on Alcohol* 2005, 66; 722-728.
5. Trevisan M, Dorn J, Falkner K, Russell M, Ram M and others: Drinking pattern and risk of non-fatal myocardial infarction: a population-based case-control study. *Addiction* 2004, 99; 313-322.
6. Laatikainen T, Manninen L, Poikolainen K and Vartiainen E: Increased mortality related to heavy alcohol intake pattern. *Journal Epidemiology and Community Health* 2003, 57; 379-384.
7. Malyutina S, Bobak M, Kurilovitch S, Gafarov V, Simonova G and others: Relation between heavy and binge drinking and all-cause and cardiovascular mortality in Novosibirsk, Russia: a prospective cohort study. *Lancet* 2002, 360; 1448-1454.
8. Hammar N, Romelsjo A and Alfredsson L: Alcohol consumption, drinking pattern and acute myocardial infarction. A case referent study based on the Swedish Twin Register. *Journal of Internal Medicine* 1997, 241; 125-131.
9. McElduff P and Dobson AJ: How much alcohol and how often? Population based case-control study of alcohol consumption and risk of a major coronary event. *British Medical Journal* 1997, 314; 1159-1164.
10. Mukamal KJ, Conigrave KM, Mittleman MA, Camargo CA, Jr., Stampfer MJ and others: Roles of drinking pattern and type of alcohol consumed in coronary heart disease in men. *New England Journal of Medicine* 2003, 348; 109-118.
11. Saccone NL, Kwon JM, Corbett J, Goate A, Rochberg N and others: A genome screen of maximum number of drinks as an alcoholism phenotype. *American Journal of Medical Genetics* 2000, 96; 632-637.
12. Osier MV, Pakstis AJ, Soodyall H, Comas D, Goldman D and others: A global perspective on genetic variation at the ADH genes reveals unusual patterns of linkage disequilibrium and diversity. *American Journal of Human Genetics* 2002, 71; 84-99.
13. Ramchandani VA, Bosron WF and Li TK: Research advances in ethanol metabolism. *Pathologie Biologie* 2001, 49; 676-682.
14. Bosron WF and Li TK: Genetic polymorphism of human liver alcohol and aldehyde dehydrogenases, and their relationship to alcohol metabolism and alcoholism. *Hepatology* 1986, 6; 502-510.

15. Vidal F, Lorenzo A, Auguet T, Olona M, Broch M and others: Genetic polymorphisms of ADH2, ADH3, CYP4502E1 Dra-I and Pst-I, and ALDH2 in Spanish men: lack of association with alcoholism and alcoholic liver disease. *Journal of Hepatology* 2004, 41; 744-750.
16. Stampfer MJ, Colditz GA, Willett WC, Speizer FE and Hennekens CH: A prospective study of moderate alcohol consumption and the risk of coronary disease and stroke in women. *New England Journal of Medicine* 1988, 319; 267-273.
17. Corrao G, Rubbiati L, Bagnardi V, Zambon A and Poikolainen K: Alcohol and coronary heart disease: a meta-analysis. *Addiction* 2000, 95; 1505-1523.
18. Gronbaek M, Becker U, Johansen D, Gottschau A, Schnohr P and others: Type of alcohol consumed and mortality from all causes, coronary heart disease, and cancer. *Annals of Internal Medicine* 2000, 133; 411-419.
19. Agarwal DP: Cardioprotective effects of light-moderate consumption of alcohol: a review of putative mechanisms. *Alcohol and Alcoholism* 2002, 37; 409-415.
20. Murray RP, Connett JE, Tyas SL, Bond R, Ekuma O and others: Alcohol volume, drinking pattern, and cardiovascular disease morbidity and mortality: is there a U-shaped function? *American Journal of Epidemiology* 2002, 155; 242-248.
21. Colditz GA, Giovannucci E, Rimm EB, Stampfer MJ, Rosner B and others: Alcohol intake in relation to diet and obesity in women and men. *American Journal of Clinical Nutrition* 1991, 54; 49-55.
22. Crouse JR, Gerson CD, DeCarli LM and Lieber CS: Role of acetate in the reduction of plasma free fatty acids produced by ethanol in man. *Journal of Lipid Research* 1968, 9; 509-512.
23. Slattery ML, McDonald A, Bild DE, Caan BJ, Hilner JE and others: Associations of body fat and its distribution with dietary intake, physical activity, alcohol, and smoking in blacks and whites. *American Journal of Clinical Nutrition* 1992, 55; 943-949.
24. Sakurai Y, Umeda T, Shintchi K, Honjo S, Wakabayashi K and others: Relation of total and beverage-specific alcohol intake to body mass index and waist-to-hip ratio: a study of self-defense officials in Japan. *European Journal of Epidemiology* 1997, 13; 893-898.
25. Rose KM, Newman B, Mayer-Davis EJ and Selby JV: Genetic and behavioral determinants of waist-hip ratio and waist circumference in women twins. *Obesity Research* 1998, 6; 383-392.
26. Haffner SM, Stern MP, Hazuda HP, Pugh J, Patterson JK and others: Upper body and centralized adiposity in Mexican Americans and non-Hispanic whites: relationship to body mass index and other behavioral and demographic variables. *International Journal of Obesity* 1986, 10; 493-502.
27. Kaye SA, Folsom AR, Prineas RJ, Potter JD and Gapstur SM: The association of body fat distribution with lifestyle and reproductive factors in a population study of postmenopausal women. *International Journal of Obesity* 1990, 14; 583-591.
28. Lakka HM, Lakka TA, Tuomilehto J and Salonen JT: Abdominal obesity is associated with increased risk of acute coronary events in men. *European Heart Journal* 2002, 23; 706-713.
29. Rexrode KM, Carey VJ, Hennekens CH, Walters EE, Colditz GA and others: Abdominal adiposity and coronary heart disease in women. *Journal of the American Medical Association* 1998, 280; 1843-1848.

30. Lissner L, Bjorkelund C, Heitmann BL, Seidell JC and Bengtsson C: Larger hip circumference independently predicts health and longevity in a Swedish female cohort. *Obesity Research* 2001, 9; 644-646.
31. Heitmann BL, Frederiksen P and Lissner L: Hip circumference and cardiovascular morbidity and mortality in men and women. *Obesity Research* 2004, 12; 482-487.
32. Thun MJ, Peto R, Lopez AD, Monaco JH, Henley SJ and others: Alcohol consumption and mortality among middle-aged and elderly U.S. adults. *New England Journal of Medicine* 1997, 337; 1705-1714.
33. Poikolainen K: Alcohol and mortality: a review. *Journal of Clinical Epidemiology* 1995, 48; 455-465.
34. White IR: The level of alcohol consumption at which all-cause mortality is least. *Journal of Clinical Epidemiology* 1999, 52; 967-975.
35. Shaper AG: Alcohol and mortality: a review of prospective studies. *British Journal of Addiction* 1990, 85; 837-847.
36. Rehm J, Greenfield TK and Rogers JD: Average volume of alcohol consumption, patterns of drinking, and all-cause mortality: results from the US National Alcohol Survey. *American Journal of Epidemiology* 2001, 153; 64-71.
37. The Copenhagen City Heart Study. Osterbroundersogelsen. A book of tables with data from the first examination (1976-78) and a five year follow-up (1981-83). The Copenhagen City Heart Study Group. *Scandinavian Journal of Social Medicine (suppl)* 1989, 41; 1-160.
38. Schnohr P, Jensen G, Lange P, Scharling H and Appleyard M: The Copenhagen City Heart Study - Østerbroundersøgelsen. Tables with data from the third examination 1991-94. *European Heart Journal (suppl)* 2001, 3; H1-H83.
39. Københavns Kommune, Statistisk Kontor: *Udenlandske Statsborgere*.  
[www.sk.kk.dk/tal\\_fakta/befolkning1d.html](http://www.sk.kk.dk/tal_fakta/befolkning1d.html)
40. Tjonneland AM and Overvad OK: [Diet, cancer and health--a population study and establishment of a biological bank in Denmark]. *Ugeskrift for Læger* 2000, 162; 350-354.
41. Overvad K, Tjonneland A, Haraldsdottir J, Ewertz M and Jensen OM: Development of a semiquantitative food frequency questionnaire to assess food, energy and nutrient intake in Denmark. *International Journal of Epidemiology* 1991, 20; 900-905.
42. Tjonneland A, Overvad K, Haraldsdottir J, Bang S, Ewertz M and others: Validation of a semiquantitative food frequency questionnaire developed in Denmark. *International Journal of Epidemiology* 1991, 20; 906-912.
43. Tunstall-Pedoe H, Kuulasmaa K, Mahonen M, Tolonen H, Ruokokoski E and others: Contribution of trends in survival and coronary-event rates to changes in coronary heart disease mortality: 10-year results from 37 WHO MONICA project populations. Monitoring trends and determinants in cardiovascular disease. *Lancet* 1999, 353; 1547-1557.
44. Gronbaek M and Heitmann BL: Validity of self-reported intakes of wine, beer and spirits in population studies. *European Journal of Clinical Nutrition* 1996, 50; 487-490.



45. Castelli WP, Doyle JT, Gordon T, Hames CG, Hjortland MC and others: Alcohol and blood lipids. The cooperative lipoprotein phenotyping study. *Lancet* 1977, 2; 153-155.
46. Szegedi A, Muller MJ, Himmerich H, Anghelescu I and Wetzel H: Carbohydrate-deficient transferrin (CDT) and HDL cholesterol (HDL) are highly correlated in male alcohol dependent patients. *Alcoholism: Clinical and Experimental Research* 2000, 24; 497-500.
47. Johansen D, Andersen PK, Jensen MK, Schnohr P and Gronbaek M: Nonlinear relation between alcohol intake and high-density lipoprotein cholesterol level: results from the Copenhagen City Heart Study. *Alcoholism: Clinical and Experimental Research* 2003, 27; 1305-1309.
48. Jørgensen HJ, Frølund C, Gustafsen J, Mosbech H, Guldhammer B and others: Registration of diagnoses in the Danish National Registry of patients. *Methods of Information in Medicine* 1986, 25 (3); 158-164.
49. Juel K and Helweg-Larsen K: The Danish registers of causes of death. *Danish Medical Bulletin* 1999, 46; 354-357.
50. Madsen M, Davidsen M, Rasmussen S, Abildstrom SZ and Osler M: The validity of the diagnosis of acute myocardial infarction in routine statistics: a comparison of mortality and hospital discharge data with the Danish MONICA registry. *Journal of Clinical Epidemiology* 2003, 56; 124-130.
51. Møller JØ, The Central Office of Civil Registration, The Danish Ministry of Interior Affairs and Health, [www.cpr.dk](http://www.cpr.dk). *Personal Communication*, April 2006.
52. Mehta CR and Patel NR: Exact logistic regression: theory and examples. *Statistics in Medicine* 1995, 14; 2143-2160.
53. Rothman K & Greenland S: Basic Methods for Sensitivity Analysis and External Adjustment. In: *Modern Epidemiology* pp. 343-357. Lippincott, Williams and Wilkins 1998.
54. Zintzaras E, Stefanidis I, Santos M and Vidal F: Do alcohol-metabolizing enzyme gene polymorphisms increase the risk of alcoholism and alcoholic liver disease? *Hepatology* 2006, 43; 352-361.
55. Sun F, Tsuritani I and Yamada Y: Contribution of genetic polymorphisms in ethanol-metabolizing enzymes to problem drinking behavior in middle-aged Japanese men. *Behavior Genetics* 2002, 32; 229-236.
56. Whitfield JB, Nightingale BN, Bucholz KK, Madden PA, Heath AC and others: ADH genotypes and alcohol use and dependence in Europeans. *Alcoholism: Clinical and Experimental Research* 1998, 22; 1463-1469.
57. Ogurtsov PP, Garmash IV, Miandina GI, Guschin AE, Itkes AV and others: Alcohol dehydrogenase ADH2-1 and ADH2-2 allelic isoforms in the Russian population correlate with type of alcoholic disease. *Addiction Biology* 2001, 6; 377-383.
58. Rodrigo L, Alvarez V, Rodriguez M, Perez R, Alvarez R and others: N-acetyltransferase-2, glutathione S-transferase M1, alcohol dehydrogenase, and cytochrome P450IIIE1 genotypes in alcoholic liver cirrhosis: a case-control study. *Scandinavian Journal of Gastroenterology* 1999, 34; 303-307.
59. Grove J, Brown AS, Daly AK, Bassendine MF, James OF and others: The RsaI polymorphism of CYP2E1 and susceptibility to alcoholic liver disease in Caucasians: effect on age of presentation and dependence on alcohol dehydrogenase genotype. *Pharmacogenetics* 1998, 8; 335-342.

60. Espinos C, Sanchez F, Ramirez C, Juan F and Najera C: Polymorphism of alcohol dehydrogenase genes in alcoholic and nonalcoholic individuals from Valencia (Spain). *Hereditas* 1997, 126; 247-253.
61. Sherman DI, Ward RJ, Yoshida A and Peters TJ: Alcohol and acetaldehyde dehydrogenase gene polymorphism and alcoholism. *Experientia Supplementum* 1994, 71; 291-300.
62. Vidal F, Perez J, Panisello J, Toda R, Gutierrez C and others: Atypical liver alcohol dehydrogenase in the Spanish population: its relation with the development of alcoholic liver disease. *Alcoholism: Clinical and Experimental Research* 1993, 17; 782-785.
63. Gilder FJ, Hodgkinson S and Murray RM: ADH and ALDH genotype profiles in Caucasians with alcohol-related problems and controls. *Addiction* 1993, 88; 383-388.
64. Poupon RE, Nalpas B, Coutelle C, Fleury B, Couzigou P and others: Polymorphism of alcohol dehydrogenase, alcohol and aldehyde dehydrogenase activities: implication in alcoholic cirrhosis in white patients. The French Group for Research on Alcohol and Liver. *Hepatology* 1992, 15; 1017-1022.
65. Day CP, Bashir R, James OF, Bassendine MF, Crabb DW and others: Investigation of the role of polymorphisms at the alcohol and aldehyde dehydrogenase loci in genetic predisposition to alcohol-related end-organ damage. *Hepatology* 1991, 14; 798-801.
66. Couzigou P, Fleury B, Groppi A, Cassaigne A, Begueret J and others: Genotyping study of alcohol dehydrogenase class I polymorphism in French patients with alcoholic cirrhosis. The French Group for Research on Alcohol and Liver. *Alcohol and Alcoholism* 1990, 25; 623-626.
67. Mukamal KJ, Jensen MK, Gronbaek M, Stampfer MJ, Manson JE and others: Drinking frequency, mediating biomarkers, and risk of myocardial infarction in women and men. *Circulation* 2005, 112; 1406-1413.
68. Mumenthaler MS, Taylor JL, O'Hara R and Yesavage JA: Gender differences in moderate drinking effects. *Alcohol Research and Health* 1999, 23; 55-64.
69. Register TC, Cline JM and Shively CA: Health issues in postmenopausal women who drink. *Alcohol Research and Health* 2002, 26; 299-307.
70. Gavalier JS, Deal SR, Van Thiel DH, Arria A and Allan MJ: Alcohol and estrogen levels in postmenopausal women: the spectrum of effect. *Alcoholism: Clinical and Experimental Research* 1993, 17; 786-790.
71. Colditz GA, Willett WC, Stampfer MJ, Rosner B, Speizer FE and others: Menopause and the risk of coronary heart disease in women. *New England Journal of Medicine* 1987, 316; 1105-1110.
72. Murray RP, Connett JE, Makela P and Rehm J: Difficulty in demonstrating a risk from drinking pattern in fourteen years of coronary heart disease morbidity and mortality: the Lung Health Study. *Addictive Behaviors* 2005, 30; 875-887.
73. Dorn JM, Hovey K, Muti P, Freudenheim JL, Russell M and others: Alcohol drinking patterns differentially affect central adiposity as measured by abdominal height in women and men. *Journal of Nutrition* 2003, 133; 2655-2662.
74. Lieber CS: Microsomal ethanol-oxidizing system (MEOS): the first 30 years (1968-1998)--a review. *Alcoholism: Clinical and Experimental Research* 1999, 23; 991-1007.

75. Lands WE and Zakhari S: The case of the missing calories. *American Journal of Clinical Nutrition* 1991, 54; 47-48.
76. Buemann B and Astrup A: How does the body deal with energy from alcohol? *Nutrition* 2001, 17; 638-641.
77. Trevisan M, Schisterman E, Mennotti A, Farchi G and Conti S: Drinking pattern and mortality: the Italian Risk Factor and Life Expectancy pooling project. *Annals of Epidemiology* 2001, 11; 312-319.
78. Cordell HJ and Clayton DG: Genetic association studies. *Lancet* 2005, 366; 1121-1131.
79. Rothman K: Biases in Study Design. In: *Epidemiology - An introduction* pp. 94-112. Oxford University Press 2002.
80. Rehm J and Gmel G: Patterns of alcohol consumption and social consequences. Results from an 8-year follow-up study in Switzerland. *Addiction* 1999, 94; 899-912.
81. Smith GD. Health Inequalities. The Policy Press (2006).
82. Rehm J, Ashley MJ, Room R, Single E, Bondy S and others: On the emerging paradigm of drinking patterns and their social and health consequences. *Addiction* 1996, 91; 1615-1621.
83. Gronbaek M, Tjonneland A, Johansen D, Stripp C and Overvad K: Type of alcohol and drinking pattern in 56, 970 Danish men and women. *European Journal of Clinical Nutrition* 2000, 54; 174-176.
84. Shaper AG, Wannamethee G and Walker M: Alcohol and mortality in British men: explaining the U-shaped curve. *Lancet* 1988, 2; 1267-1273.
85. Rothman K & Greenland S: Precision and Validity in Epidemiological Studies. In: *Modern Epidemiology* pp. 115-134. Lippincott, Williams and Wilkins 1998.
86. Hernan MA, Hernandez-Diaz S and Robins JM: A structural approach to selection bias. *Epidemiology* 2004, 15; 615-625.
87. Smith GD and Ebrahim S: Mendelian randomization: prospects, potentials, and limitations. *International Journal of Epidemiology* 2004, 33; 30-42.
88. Savitz DA: Selection Bias in Cohort Studies. In: *Interpreting Epidemiologic Evidence* pp. 51-80. Oxford University Press 2003.



## PAPERS

## **Alcoholism and alcohol drinking habits predicted from alcohol dehydrogenase genes**

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## SUMMARY

**Background** Alcoholism and alcohol drinking habits are partly genetically determined. Because alcohol is degraded mainly by liver alcohol dehydrogenase (ADH), well-known functional variation in *ADH2* and *ADH3* genes may influence risk of alcoholism and be associated with alcohol drinking habits.

**Methods** We genotyped 9080 men and women from the general Danish population for *ADH2* and *ADH3* polymorphisms. Alcohol drinking habits were assessed from questionnaire and information on alcoholism was assessed from the *Short Michigan Alcoholism Screening Test* and from hospital registry information.

**Findings** Men with *ADH2* slow versus fast alcohol degradation had a higher alcohol intake (relative alcohol intake 1.3 [95% confidence interval 1.2-1.5]), were more often daily drinkers (odds ratio 2.5 [1.5-4.1]), heavy drinkers (OR 3.1 [1.7-5.7]), excessive drinkers (OR 2.7 [1.1-6.5] and had higher risk of alcoholism (OR 4.8 [1.5-15]). Furthermore, men with *ADH3* intermediate and slow versus fast alcohol degradation were more often heavy drinkers (OR 1.4 [1.1-1.8] and 1.4 [1.0-1.9]) and excessive drinkers (OR 1.6 [1.1-2.3] and 1.7 [1.1-2.6]). Results for women were similar. Finally, because slow *ADH2* alcohol degradation is found in >90% of Caucasians compared with <10% of East Asians, the population attributable risk of heavy alcohol drinking and alcoholism by *ADH2*: slow alcohol degradation is 67% and 70% among Caucasians compared with 9% and 24% among East Asians.

**Interpretation** Our results strongly suggest that alcoholism and alcohol drinking habits are partly determined from *ADH2* and *ADH3* genotypes.

## INTRODUCTION

Alcoholism and alcohol drinking in general represent huge public health problems in most countries worldwide, preventing many individuals from successfully holding a job or looking after a family. In addition, excessive use of alcohol leads to diseases such as liver cirrhosis, chronic pancreatitis, upper gastrointestinal cancers, cardiomyopathy, polyneuropathy and dementia. It has been shown in twin studies that approximately 50 percent of alcoholism and problem drinking in Caucasians can be explained by heritability.<sup>1,2</sup> Only a few genes like the *ADH* and *acetaldehyde dehydrogenase* genes have been directly implicated in explaining alcoholism and/or alcohol drinking habits.<sup>3-7</sup>

The *ADH* gene cluster is situated on chromosome 4. Although this region is known from whole genome scans to be associated with alcohol drinking habits and alcoholism,<sup>8</sup> it is only sparsely studied among Caucasians whether known functional *ADH2* and *ADH3* polymorphisms are associated with alcoholism and alcohol drinking behaviour,<sup>9</sup> and mainly in small case-control studies of men.<sup>10</sup> This is biologically plausible, because the *ADH2*·2 versus the *ADH2*·1 allele confer a 38 fold increase in maximal alcohol degradation rate, and because the *ADH3*·1 versus the *ADH3*·2 allele confer a 2.5 fold increase in maximal alcohol degradation rate.<sup>11</sup> During normal alcohol degradation, the product acetaldehyde is only found in low concentrations in the body. When concentrations of acetaldehyde become high, e.g. during treatment with disulfiram (used in some countries to prevent alcohol intake) or in individuals with a defective acetaldehyde dehydrogenase (found among Asians), individuals experience severe nausea and flushing and automatically abstain from drinking alcohol. Therefore, compared with fast alcohol degradation, it is indeed possible that individuals carrying the slow alcohol degradation *ADH2*·1 and *ADH3*·2 alleles are able to drink large quantities of alcohol without experiencing discomfort due to elevated acetaldehyde levels, and consequently are more likely to use alcohol excessively and to develop alcoholism.

In the present study of a Caucasian general population with similar numbers of men and women, we tested the hypothesis that slow alcohol degradation *ADH2*·1 and *ADH3*·2 alleles are associated with adverse alcohol drinking habits and increased risk of alcoholism.



## METHODS

### *Study population*

Our data originates from The Copenhagen City Heart Study, which is a series of studies conducted in the Danish general population. Examinations consisted of interview, physical examination, and more especially, blood was given for DNA purification at the 1991-94 examination. All participants gave written consent and the ethics committee for Copenhagen and Frederiksberg approved the study (no. 100.2039/91). Enrolment and examination procedures have been described in more detail elsewhere.<sup>12,13</sup> Of the 17,180 individuals invited to the 1991-94 examination, 10,135 participated, 9,259 gave blood and 9,222 were successfully genotyped for *ADH2* and *ADH3* polymorphisms. Participants of non-Danish descent were excluded (n=142). In all, 9080 individuals were eligible for analyses, some of whom also participated in the examinations in 1981-83 (n=6615) and in 2001-03 (n=4684).

### *Genotyping procedures*

The *ADH2*·2 allele (Arg47His in exon 3) and *ADH3*·2 allele (Iso349Val in exon 8) were identified by means of duplex polymerase chain reaction (PCR) followed by Nanogen microelectronic chip technology (Nanogen NMW 1000 Nanochip<sup>TM</sup> Molecular Biology Workstation)<sup>14</sup> using standard conditions (details available from authors). In short, two DNA products of 324 and 442 basepairs were produced by duplex PCR, loaded on microchips and incubated with reporters (fluorescence labelled oligonucleotides whose sequences are complementary to the specific alleles). Subsequently, the wavelength of light emission upon energy transfer (heating) was measured, identifying the specific hybridised reporter and hence the genotype. In a validation study, the accuracy of the Nanogen method was found to be comparable to restriction fragment length polymorphism.<sup>15</sup>

### *Endpoints*

Questions on drinking habits were included in the questionnaire at the examinations in 1981-83, 1991-94 and 2001-03. Amount of alcohol intake was reported as usual intake of weekly beers, wine, and spirits. Assuming one drink to be equal to 12 grams of pure alcohol, a measure of total weekly intake was calculated. We defined heavy drinking as drinking more than 21 drinks/week for men and 14 drinks/week for women, and excessive drinking as drinking more than 35 drinks/week for men and 21 drinks/week for women.<sup>16</sup> Participants were defined as daily drinkers if they reported to drink alcohol every day.

We defined alcoholism from questionnaire as well as from hospital discharge information. The former definition was taken from the 1991-1994 questionnaire, which included a screening test for alcoholism (10 question version of the *Short Michigan Alcoholism Screening Test*<sup>17</sup> (SMAST)). The test included questions such as ‘Do you feel you are a normal drinker?’ and ‘Have you ever gone to anyone for help about your drinking?’. Each affirmative response scored one point. Information on hospitalisations for alcoholism was obtained from the Danish Hospital Discharge Register where all hospitalisations in Denmark are registered.<sup>18</sup> Diagnoses are classified according to the World Health Organisation’s International

Classification of Diseases (ICD), 8th and 10th revision. Diagnoses suggestive of alcoholism (including diagnoses for alcohol psychosis and alcohol intoxication) were used as endpoints (ICD-8 codes 291.09-291.99, 303.09-303.99, N980.09-980.99, E860, and ICD-10 codes F10.0-F10.9).

### *Statistical analyses*

All statistical models included *ADH2* and *ADH3* genotypes, age and years of school education using the SAS/Stat software (version 8.02). *ADH2*·2 heterozygotes were combined with *ADH2*·2 homozygotes (n=6).

Estimated haplotype frequencies were calculated by Hplus.<sup>19,20</sup> Linkage disequilibrium was expressed as  $r^2$  and  $D'$ .<sup>21,22</sup>

To study the association between *ADH2* and *ADH3* genotypes and amount of alcohol intake, the total weekly intake was used on a continuous scale in one analysis and dichotomised in other analyses. For the former purpose, the correlated mixed distribution model was applied (*Mixcorr* macro<sup>23</sup>). This model handles data with clumping at zero and a lognormal distribution for non-zero values, and contains components to model the probability of a non-zero value and the mean of non-zero values (=any alcohol intake), allowing for repeated measurements using random effects and allowing for correlation between the two components.<sup>23</sup> This means that if a variable affects the mean amount by affecting both the probability of occurrence of a non-zero value and also the mean of a non-zero value, these effects can be separated and quantified.

To study the association between *ADH2* and *ADH3* genotypes and daily, heavy and excessive drinking, we applied logistic regression in which a random intercept was included to induce a compound symmetry covariance structure for individuals with repeated measurements (proc nlmixed). We applied unconditional logistic regression to study associations between *ADH2* and *ADH3* genotypes and dichotomised SMAST score (total score  $\geq 1$  point, and total score  $\geq 3$  points, respectively) (proc genmod).

Risk estimates for alcoholism defined from hospitalisations were computed by means of Cox proportional hazard regression (proc phreg). Age was used as the time axis and analyses were corrected for delayed entry. Vital status of the participants was obtained from the National Central Person Register. The observation time for each participant was the period from participation in the Copenhagen City Heart Study, until date of alcoholism, death, emigration outside Denmark, or January 1, 2004, whichever came first. We had 100% follow-up.

Population attributable risk was calculated as [proportion of exposed in the population·(odds ratio-1)]/[proportion of exposed in the population·(odds ratio-1)+1].<sup>24</sup>

## RESULTS

The frequencies of the *ADH2* and *ADH3* alleles coding for slow alcohol degradation was 0.98 (*ADH2*·1) and 0.42 (*ADH3*·2) (table 1). Genotypes were in Hardy-Weinberg equilibrium ( $P=0.8$  for *ADH2* genotypes and  $P=0.7$  for *ADH3* genotypes by  $\chi^2$  test). There was a high degree of association between the *ADH2*·1 and *ADH3*·2 alleles, both coding for the slow alcohol degradation enzymatic forms (linkage disequilibrium coefficients  $D'=0.90$  and  $r^2=0.012$ ).

For *ADH2*, we found that alcohol drinking men and women who were homozygous for the slow alcohol degrading *ADH2*·1 allele had a more than 30% higher alcohol intake than alcohol drinking men and women who were fast alcohol degrading *ADH2*·2 heterozygotes or homozygotes (relative alcohol intake 1.3 [95% CI 1.2-1.5] men; 1.3 [1.2-1.5] women) (table 2). Furthermore, odds for any, daily, heavy and excessive alcohol drinking were 2-4 times higher among *ADH2*·1 homozygotes than among *ADH2*·2 heterozygotes and homozygotes combined. Using the *Short Michigan Alcoholism Screening Test* (SMAST) we found that men, who were homozygous for the slow alcohol degradation *ADH2*·1 allele, had a 2-5 fold risk of alcoholism compared with men with the fast alcohol degradation *ADH2*·2/1 or *ADH2*·2/2 genotypes (table 2). In addition, there was a trend towards an elevated hazard ratio of hospitalisation for alcoholism in men and women with the slow alcohol degradation *ADH2*·1/1 genotype (hazard ratio 2.4 [0.9-6.5] men; 3.6 [0.5-26] women).

For *ADH3*, odds for heavy and excessive alcohol drinking were 40% to 70% higher among men who were heterozygous or homozygous for the slow alcohol degrading *ADH3*·2 allele than among men who were homozygous for the fast alcohol degrading *ADH3*·1 allele (table 3). Similar results were found in women; however, the effects were slightly smaller and only statistically significant for heavy drinking. *ADH3* genotype was not associated with alcoholism (table 3).

Because of linkage disequilibrium between the *ADH2*·1 and *ADH3*·2 alleles, and the relatively large effect on enzyme activity of the *ADH2* polymorphism, our results for *ADH3* could be influenced by *ADH2* genotype. Therefore, *ADH3* analyses were repeated solely on individuals who were *ADH2*·1 homozygotes (95.5 % of the study cohort): we found similar results, indicating that the effect of *ADH3* genotype was independent of *ADH2* genotype (data not shown).

We also performed analyses on genotype combinations, ranking genotypes in order of expected total enzyme activity, and tested for linear trend in each of the variables for alcohol drinking habits and alcoholism (figure 1). For 7 of 8 endpoints, there was a statistically significant trend test in the expected direction: individuals with slow versus fast alcohol degradation drank more alcohol and more often had a higher risk of alcoholism.

*ADH2* genotypes are very differently distributed among Caucasians and East Asians (figure 2). Among Caucasians, >90% carry the *ADH2*·1/1 genotype, coding for slow alcohol degradation, whereas among East Asians <10% carry this genotype. Hence, the population attributable risk of heavy drinking and alcoholism according to the *ADH2*·1/1 genotype is 67% and 70% among Caucasians compared with 9% and 24% among East Asians.

## DISCUSSION

Our results strongly suggest that alcoholism and alcohol drinking habits are partly determined from *ADH2* and *ADH3* genotypes. Men and women with *ADH2* slow versus fast alcohol degradation drink more alcohol, are more often daily, heavy and excessive drinkers and have higher risk of alcoholism, and men and women with *ADH3* intermediate and slow versus fast alcohol degradation are more often heavy and excessive drinkers. Our results also suggest that *ADH2* and *ADH3* genotypes may partly explain why Caucasians generally drink more alcohol than East Asians. The population risk of heavy drinking and alcoholism attributed to the *ADH2*·1/1 genotype was 67% and 70% among Caucasians in the present study, but only 9% and 24% among East Asians.

Among men, we found relative estimates for alcoholism on 2.1 to 4.8 among *ADH2*·1 homozygotes, which is comparable to results from a recent meta-analysis consisting predominantly of East Asian studies.<sup>10</sup> Also, the odds ratio on 3 for heavy drinking for men agrees with what was previously found among Asian men.<sup>25</sup> Separate estimates for women were not available for any of the endpoints in previous studies. To our knowledge, no previous studies have investigated associations between *ADH3* and alcohol drinking habits.

A likely explanation for our findings is that the differences in enzyme activity from the *ADH2* and *ADH3* polymorphisms result in intra-individual differences in alcohol degradation and that, for a given level of alcohol intake, individuals with fast alcohol degradation have higher levels of acetaldehyde and thus more unpleasant symptoms compared with individuals with slow alcohol degradation. However, an effect of *ADH2* and *ADH3* polymorphisms on alcohol degradation *in vivo* has been difficult to prove, maybe because of insensitive methods.<sup>26</sup> Alternatively, acetaldehyde stemming from extrahepatic ethanol metabolism by *ADH2* and *ADH3* situated in skin and blood vessels may cause local effects, such as flushing, and individuals with the most active enzymes may have more unpleasant symptoms when drinking alcohol than individuals with the less active enzymatic forms.

Our study had several strengths. First of all, sample size is large and provided adequate power to study associations between *ADH2*·1/2, that is rare among Caucasian populations, and several endpoints and to detect associations with *ADH3*, which had minor effects on the studied endpoints than *ADH2*. Furthermore, participants were men and women all from the general population of Danish descent. Hence, population stratification is unlikely to have affected our results. Alcohol drinking habits were described in several dimensions and information on alcoholism was obtained from two independent sources (questionnaire and hospital registry information). All endpoints were assessed independently from genotyping and participants were unaware of the purpose of this study when enrolled.

In conclusion, our data strongly suggest that alcoholism and alcohol drinking habits are partly determined from *ADH2* and *ADH3* genotypes. Results for men and women were comparable and, as expected, effects of *ADH2* were larger than effects of *ADH3*.

## References

1. Han C, McGue MK, Iacono WG. Lifetime tobacco, alcohol and other substance use in adolescent Minnesota twins: univariate and multivariate behavioral genetic analyses. *Addiction* 1999; **94**: 981-93.
2. Kendler KS, Neale MC, Heath AC, et al. A twin-family study of alcoholism in women. *Am J Psychiatry* 1994; **151**: 707-15.
3. Tanaka F, Shiratori Y, Yokosuka O, et al. Polymorphism of alcohol-metabolizing genes affects drinking behavior and alcoholic liver disease in Japanese men. *Alcohol Clin Exp Res* 1997; **21**: 596-601.
4. Okamoto K, Murawaki Y, Yuasa A, I, et al. Effect of ALDH2 and CYP2E1 gene polymorphisms on drinking behavior and alcoholic liver disease in Japanese male workers. *Alcohol Clin Exp Res* 2001; **25**: 19S-23S.
5. Maezawa Y, Yamauchi M, Toda G, et al. Alcohol-metabolizing enzyme polymorphisms and alcoholism in Japan. *Alcohol Clin Exp Res* 1995; **19**: 951-4.
6. Chen CC, Lu RB, Chen YC, et al. Interaction between the functional polymorphisms of the alcohol-metabolism genes in protection against alcoholism. *Am J Hum Genet* 1999; **65**: 795-807.
7. Sun F, Tsuritani I, Honda R, et al. Association of genetic polymorphisms of alcohol-metabolizing enzymes with excessive alcohol consumption in Japanese men. *Human Genetics* 1999; **105**: 295-300.
8. Saccone NL, Kwon JM, Corbett J, et al. A genome screen of maximum number of drinks as an alcoholism phenotype. *Am J Med Genet* 2000; **96**: 632-7.
9. Whitfield JB, Nightingale BN, Bucholz KK, et al. ADH genotypes and alcohol use and dependence in Europeans. *Alcohol Clin Exp Res* 1998; **22**: 1463-9.
10. Zintzaras E, Stefanidis I, Santos M, et al. Do alcohol-metabolizing enzyme gene polymorphisms increase the risk of alcoholism and alcoholic liver disease? *Hepatology* 2006; **43**: 352-61.
11. Bosron WF, Li TK. Genetic polymorphism of human liver alcohol and aldehyde dehydrogenases, and their relationship to alcohol metabolism and alcoholism. *Hepatology* 1986; **6**: 502-10.
12. The Copenhagen City Heart Study. Osterbroundersogelsen. A book of tables with data from the first examination (1976-78) and a five year follow-up (1981-83). The Copenhagen City Heart Study Group. *Scand J Soc Med (suppl)* 1989; **41**: 1-160.
13. Schnohr P, Jensen G, Lange P, et al. The Copenhagen City Heart Study - Østerbroundersøgelsen. Tables with data from the third examination 1991-94. *Eur Heart J (suppl)* 2001; **3**: H1-H83.
14. Sosnowski RG, Tu E, Butler WF, et al. Rapid determination of single base mismatch mutations in DNA hybrids by direct electric field control. *Proc Natl Acad Sci USA* 1997; **94**: 1119-23.
15. Sethi AA, Tybjaerg-Hansen A, Andersen RV, et al. Nanogen microelectronic chip for large-scale genotyping. *Clin Chem* 2004; **50**: 443-6.
16. Jensen MK, Sorensen TI, Andersen AT, et al. A prospective study of the association between smoking and later alcohol drinking in the general population. *Addiction* 2003; **98**: 355-63.
17. Seltzer M, Vinokur A, Van Rooijen LJ. A self-administered Short Michigan Alcohol Screening Test (SMAST). *J Stud Alcohol* 1975; **36**: 117-26.

18. Jørgensen HJ, Frølund C, Gustafsen J, et al. Registration of diagnoses in the Danish National Registry of patients. *Meth Inf Med* 1986; **25** (3): 158-64.
19. Li, S, Laws, R. J., and Zhao, L. P. HPlus. (2.1). 1-1-2003. Fred Hutchinson Cancer Research Center, Seattle WA 98109.
20. Li SS, Khalid N, Carlson C, et al. Estimating haplotype frequencies and standard errors for multiple single nucleotide polymorphisms. *Biostatistics* 2003; **4**: 513-22.
21. Lewontin RC. The Interaction of selection and linkage.I. General considerations: heterotic models. *Genetics* 1964; **49**: 49-67.
22. Pritchard JK, Przeworski M. Linkage disequilibrium in humans: models and data. *Am J Hum Genet* 2001; **69**: 1-14.
23. Tooze JA, Grunwald GK, Jones RH. Analysis of repeated measures data with clumping at zero. *Stat Methods Med Res* 2002; **11**: 341-55.
24. Walter SD. Calculation of attributable risks from epidemiological data. *Int J Epidemiol* 1978; **7**: 175-82.
25. Sun F, Tsuritani I, Yamada Y. Contribution of genetic polymorphisms in ethanol-metabolizing enzymes to problem drinking behavior in middle-aged Japanese men. *Behavior Genetics* 2002; **32**: 229-36.
26. Neumark YD, Friedlander Y, Durst R, et al. Alcohol dehydrogenase polymorphisms influence alcohol-elimination rates in a male Jewish population. *Alcohol Clin Exp Res* 2004; **28**: 10-4.

Table 1: **Distribution of *ADH2* and *ADH3* genotypes.**

	<i>ADH3</i>					
	Men			Women		
	1/1	1/2	2/2	1/1	1/2	2/2
	(fast)	(intermediate)	(slow)	(fast)	(intermediate)	(slow)
<i>ADH2</i>						
1/1 (slow)	1272	1882	697	1570	2347	907
1/2 (intermediate)	110	70	5	130	81	3
2/2 (fast)	2	1	0	3	0	0

Table 2: Association between *ADH2* genotype and alcohol drinking habits and alcoholism

	MEN		WOMEN	
Genotype	<i>ADH2</i> : 1/2+2/2	<i>ADH2</i> : 1/1	<i>ADH2</i> : 1/2+2/2	<i>ADH2</i> : 1/1
Alcohol degradation	Fast	Slow	Fast	Slow
Number of participants	188	3851	217	4824
<b>Alcohol drinking habits</b>				
Weekly alcohol intake* (N: 3784 M, 4052 W)	1.0	1.3 (1.2-1.5)	1.0	1.3 (1.2-1.5)
Any alcohol intake† (N: 3784 M, 4052 W)	1.0	2.1 (1.0-4.5)	1.0	3.1 (1.8-5.3)
Daily drinking† (N: 2015 M, 1259 W)	1.0	2.5 (1.5-4.1)	1.0	1.9 (1.1-3.5)
Heavy drinking† (N: 1262 M, 814 W)	1.0	3.1 (1.7-5.7)	1.0	3.0 (1.4-6.4)
Excessive drinking† (N: 507 M, 353 W)	1.0	2.7 (1.1-6.5)	1.0	4.2 (1.3-13)
<b>Alcoholism</b>				
SMAST score ≥1 point† (N: 1076 M, 597 W)	1.0	2.1 (1.4-3.1)	1.0	0.9 (0.6-1.4)
SMAST score ≥3 point† (N: 290 M, 65 W)	1.0	4.8 (1.5-15)	1.0	1.6 (0.4-6.6)
Hospitalisation‡ (N: 209 M, 90 W)	1.0	2.4 (0.9-6.5)	1.0	3.6 (0.5-26)

Note: Heavy drinking was defined as drinking more than 21 drinks/week for men and 14 drinks/week for women, and excessive drinking as drinking more than 35 drinks/week for men and 21 drinks/week for women. N indicates the number of men (M) and women (W) who are cases in the different analyses. Adjustment was made for *ADH3*-genotype, age, years of school education, and examination year. SMAST: Short Michigan Alcoholism Screening Test. \* Shown numbers are relative alcohol intake (95% confidence intervals). † Shown numbers are odds ratios (95% confidence intervals). ‡ Shown numbers are hazard ratios (95% confidence intervals).

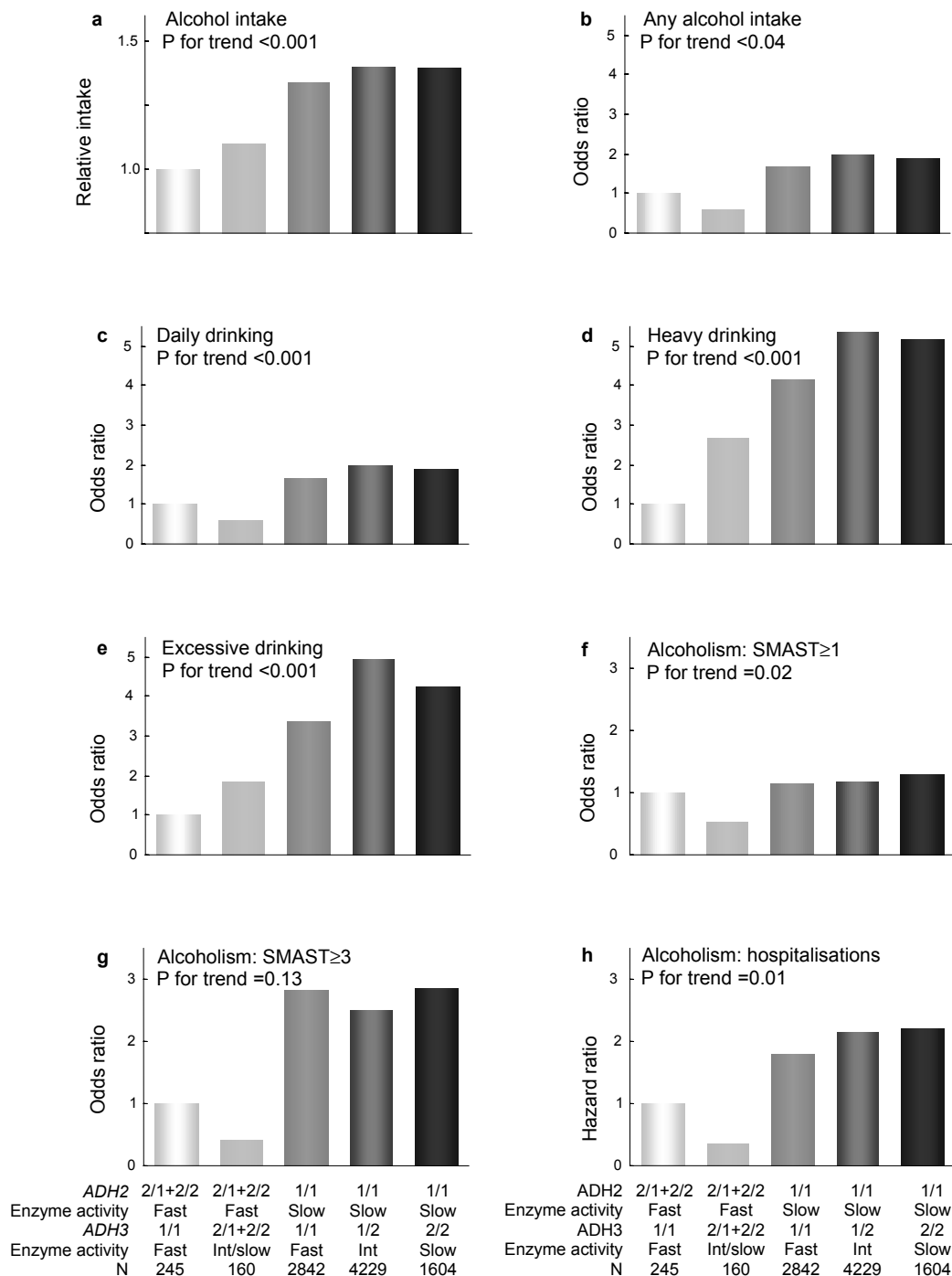


Table 3. **Association between *ADH3* genotype and alcohol drinking habits and alcoholism**

	MEN			WOMEN		
Genotype	<i>ADH3</i> -1/1	<i>ADH3</i> -1/2	<i>ADH3</i> -2/2	<i>ADH3</i> -1/1	<i>ADH3</i> -1/2	<i>ADH3</i> -2/2
Alcohol degradation	Fast	Intermediate	Slow	Fast	Intermediate	Slow
Number of participants	1384	1953	702	1703	2428	910
<b>Alcohol drinking habits</b>						
Weekly alcohol intake* (N: 3784 M, 4052 W)	1.00	1.1 (1.0-1.1)	1.1 (1.0-1.2)	1.00	1.0 (1.0-1.1)	1.0 (1.0-1.1)
Any alcohol intake† (N: 3784 M, 4052 W)	1.0	1.3 (0.9-1.8)	0.7 (0.5-1.2)	1.0	1.1 (0.9-1.4)	1.0 (0.7-1.4)
Daily drinking† (N: 2015 M, 1259 W)	1.0	1.1 (0.9-1.4)	1.1 (0.8-1.5)	1.0	1.2 (0.9-1.5)	1.2 (0.9-1.7)
Heavy drinking† (N: 1262 M, 814 W)	1.0	1.4 (1.1-1.8)	1.4 (1.0-1.9)	1.0	1.3 (1.0-1.7)	1.2 (1.0-1.7)
Excessive drinking† (N: 507 M, 353 W)	1.0	1.6 (1.1-2.3)	1.7 (1.1-2.6)	1.0	1.4 (0.9-2.0)	1.0 (0.6-1.6)
<b>Alcoholism</b>						
SMAST score ≥1 point† (N: 1076 M, 597 W)	1.0	1.1 (0.9-1.2)	1.1 (0.9-1.3)	1.0	0.9 (0.8-1.1)	1.1 (0.8-1.4)
SMAST score ≥3 point† (N: 290 M, 65 W)	1.0	1.0 (0.7-1.3)	1.1 (0.8-1.5)	1.0	0.6 (0.4-1.1)	0.8 (0.4-1.5)
Hospitalisation‡ (N: 209 M, 90 W)	1.0	1.2 (0.9-1.6)	1.1 (0.7-1.6)	1.0	1.2 (0.8-2.1)	1.6 (0.9-2.9)

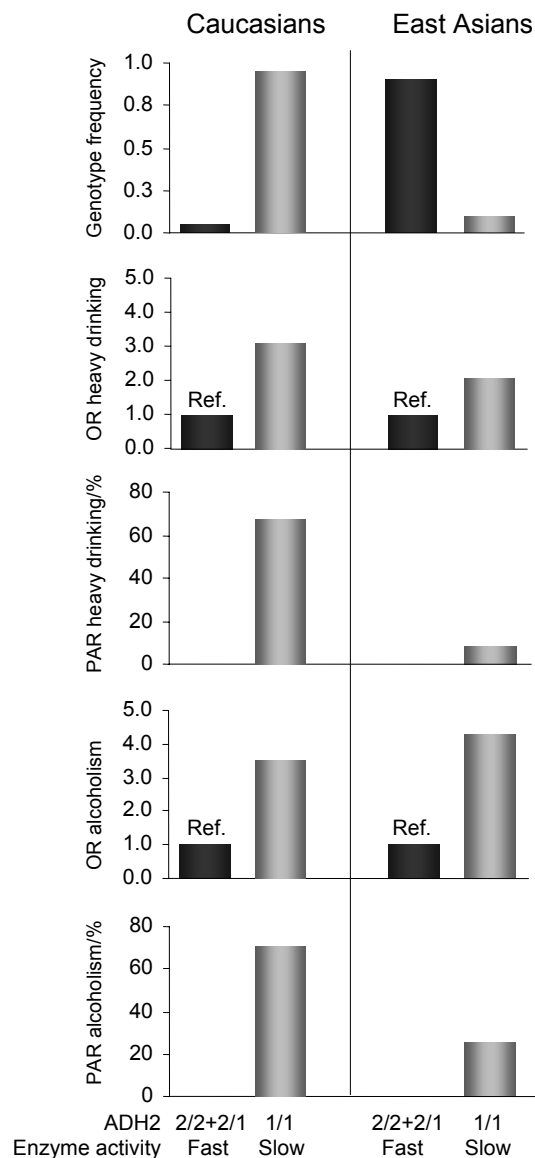
Note: Heavy drinking was defined as drinking more than 21 drinks/week for men and 14 drinks/week for women, and excessive drinking as drinking more than 35 drinks/week for men and 21 drinks/week for women. N indicates the number of men (M) and women (W) who are cases in the different analyses. Adjustment was made for *ADH2*-genotype, age, years of school education and examination year. SMAST: Short Michigan Alcoholism Screening Test. \* Shown numbers are relative alcohol intakes (95% confidence intervals). † Shown numbers are odds ratios (95% confidence intervals). ‡ Shown numbers are hazard ratios (95% confidence intervals)

**Figure 1:** Association between the combined *ADH2* and *ADH3* genotypes and alcohol drinking habits and alcoholism



LEGEND: The combined *ADH2* and *ADH3* genotypes are ranked according to expected enzyme activity ([*ADH2*·2/1+*ADH2*·2/2, *ADH3*·1/1] > [*ADH2*·2/1+*ADH2*·2/2, *ADH3*·2/1+*ADH3*·2/2] > [*ADH2*·1/1, *ADH3*·1/1] > [*ADH2*·1/1, *ADH3*·1/2] > [*ADH2*·1/1, *ADH3*·2/2]). *ADH3*·1/2 and *ADH3*·2/2 were combined because of too few subjects in these categories among the *ADH2*·2 hetero- and homozygotes. Analyses are for men and women combined and P-values are for linear trend tests.

Figure 2. **Genotype frequencies, odds ratios and population attributable risks of heavy alcohol drinking and alcoholism according to *ADH2* genotype among Caucasians and East Asians**



LEGEND: Slow alcohol degradation *ADH2*·1/1 genotype is predominant among Caucasians, but rare among East Asians. Risks of heavy drinking and alcoholism according to *ADH2*·1/1 among Caucasians and East Asians are comparable, but because of the different genotype distributions in the two populations, population attributable risks are much higher among Caucasians. For East Asians, genotype frequencies, odds ratios and population attributable risks are calculated from previous studies.<sup>10,25</sup> Ref: Reference group, OR: Odds ratio, PAR: Population attributable risk.

# Research

## Prospective study of alcohol drinking patterns and coronary heart disease in women and men

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### Abstract

**Objective** To determine the association between alcohol drinking patterns and risk of coronary heart disease in women and men.

**Design** Population based cohort study.

**Setting** Denmark, 1993-2002.

**Participants** 28 448 women and 25 052 men aged 50-65 years, who were free of cardiovascular disease at entry to the study.

**Main outcome measures** Incidence of coronary heart disease occurring during a median follow-up period of 5.7 years.

**Results** 749 and 1283 coronary heart disease events occurred among women and men. Women who drank alcohol on at least one day a week had a lower risk of coronary heart disease than women who drank alcohol on less than one day a week. Little difference was found, however, between drinking frequency: one day a week (hazard ratio 0.64, 95% confidence interval 0.51 to 0.81), 2-4 days a week (0.63, 0.52 to 0.77), five or six days a week (0.79, 0.61 to 1.03), and seven days a week (0.65, 0.51 to 0.84). For men an inverse association was found between drinking frequency and risk of coronary heart disease across the entire range of drinking frequencies. The lowest risk was observed among men who drank daily (0.59, 0.48 to 0.71) compared with men who drank alcohol on less than one day a week.

**Conclusions** Among women alcohol intake may be the primary determinant of the inverse association between drinking alcohol and risk of coronary heart disease whereas among men, drinking frequency, not alcohol intake, seems more important.

### Introduction

Prospective studies have consistently reported a lower risk of coronary heart disease among consumers of moderate alcohol compared with abstainers.<sup>1</sup> A few studies have investigated this association by also including various measures of alcohol drinking patterns. Results consistently imply that the pattern of drinking is important and that steady drinking is more beneficial than drinking in binges.<sup>2-6</sup> In a recent such study among men it was suggested that drinking frequency is the primary determinant of the inverse association between alcohol intake and coronary heart disease, and that alcohol intake is of minor importance.<sup>6</sup> Some issues still warrant consideration however; most importantly, data on the importance of drinking patterns among women are limited and results obtained among men may not apply to women for different reasons. Firstly, sex differences in alcohol pharmacokinetics have been reported, suggesting that men have more efficient first pass metabolisms than women

whereas women may eliminate alcohol faster than men.<sup>7</sup> Secondly, oestrogen has beneficial effects on the cardiovascular system, and studies have suggested that alcohol increases oestrogen levels.<sup>8</sup>

We determined the association between alcohol drinking patterns and coronary heart disease among men and women participating in a population based cohort study consisting of middle aged Danish citizens.

### Methods

From December 1993 to May 1997, 160 725 Danish men and women were invited to participate in the diet, cancer, and health study.<sup>9</sup> Eligible cohort members were born in Denmark and had no previous cancers. Overall, 27 178 men and 29 875 women agreed to participate (response rate 35%). A detailed food frequency questionnaire consisting of 192 items was enclosed with the invitation.<sup>10 11</sup> This questionnaire was checked by an interviewer during a clinic visit, when another questionnaire concerning lifestyle and background factors was completed.

In the food frequency questionnaire alcohol intake was reported as the average amount over the preceding year. Total intake was calculated and converted into number of standard drinks, defined as containing 12 g of ethanol. Drinking frequency was reported in the background questionnaire in predefined categories (never, less than once a month, 1-3 times monthly, once a week, 2-4 times weekly, 5 or 6 times weekly, and daily). We defined abstainers as those who reported no alcohol intake (amount) and no drinking occasions (frequency). To increase homogeneity among abstainers we excluded 786 people who reported no amount but a frequency greater than zero (or vice versa). We also excluded people with missing information ( $n = 303$ ) or with conflicting answers on amount and frequency of alcohol intake ( $n = 97$ ). In all, 53 500 people were eligible for this study.

### Follow-up

We obtained information on coronary heart disease from the Danish Hospital Discharge Register<sup>12</sup> and from the Danish Register of Causes of Death,<sup>13</sup> where, respectively, all admissions to hospital for somatic conditions and causes of death in Denmark are registered. The hospital register is updated to 2002, whereas the causes of death register, which contains information on fatal incidents of coronary heart disease, is updated to 2000. In the period that was covered by both registers, the causes of death register contributed information on only 8% of cases. Hence we decided to end follow-up at January 2002, being aware that information on some fatal cases would be missed from January 2000 to January 2002.

In both registers diagnoses are classified according to the international classification of diseases, eighth and 10th revisions (codes for coronary heart disease: ICD-8, 410-414 and ICD-10, I20-I25). We obtained vital status of the participants from the National Central Person Register. To minimise the risk of including preclinical cases, we excluded 2367 participants who, at baseline, were registered with any cardiovascular disease (ischaemic stroke, arrhythmias, congestive heart failure, or peripheral arteriosclerosis).

We observed participants from enrolment until date of coronary heart event ( $n=2113$ ), death from other causes ( $n=1483$ ), emigration ( $n=183$ ), loss to follow-up ( $n=3$ ), or 1 January 2002, whichever came first.

### Statistical analysis

We calculated risk estimates using Cox proportional hazard regression models, with delayed entry implemented (SAS/STAT program software). To ensure maximal adjustment for confounding by age we used age as the time axis. We adjusted the risk estimates for known risk factors for coronary heart disease: length of school education (short,  $\leq 7$  years; medium, 8-10 years; long,  $\geq 11$  years); smoking (never; former; current, 1-14, 15-24, or  $> 24$  g of tobacco/day); physical activity during leisure time (dummy variables were coded for each of the following activities: sports, walking, bicycling, housework, gardening, do it yourself); body mass index (modelled as linear splines, with knots set at 20 and 25); total intake of fruit, vegetables, and fish; and percentage of total energy intake from saturated fat (all as continuous variables). We calculated the total intake of different dietary factors using the software program Food Calc (release 1.3, www.FoodCalc.dk). Using linear splines with knots set at quintiles of the covariate in question we evaluated the assumed linearity of quantitative risk factors. We tested assumptions of the proportional hazards model but detected no violations.

To test for linear trends we treated the median value within categories continuously. We did not include abstainers when testing for trend because they may have different trait and health status than people who consume alcohol lightly to moderately,<sup>14</sup> causing a spuriously high relative risk in this category.

To examine the magnitude of rank correlation between drinking frequency and amount of alcohol, we calculated Spearman's correlation coefficient. We tested the interaction between sex and drinking frequency using a nested log likelihood test where we compared a model containing the variables as single terms with a model including the interaction terms.

To tackle the external validity of our results, we compared the observed with the expected number of cases, on the basis of age and calendar year specific incidence rates in the general Danish population. These were calculated using nationwide information from the Danish Hospital Discharge Register.<sup>12</sup>

## Results

Overall, 53 500 people were eligible for our study: 28 448 women and 25 052 men. Women consumed a median of 5.5 alcoholic drinks a week (fifth to 95th centiles, 0.3-24) and men 11.3 (1.1-47). Drinking frequency was highly correlated with amount of alcohol intake among both women and men ( $r=0.86$  and  $r=0.78$ ).

Infrequent drinkers (less than one day a week) and daily drinkers (daily) were more likely to be smokers, to have a lower intake of fruit and vegetables, and to be less educated than participants in the in between drinking frequencies (table 1). Body mass index was inversely associated with drinking frequency and frequent drinkers had the lowest body mass index. These trends

were the same for both sexes. Generally, fewer women than men were current and heavy smokers ( $> 25$  g of tobacco daily) and women had more hours of physical activity a week and consumed more fruit and vegetables.

During follow-up (median 5.7 years, range 0.01-8.10) 749 women and 1283 men developed coronary heart disease. Information on 1933 of these cases came from the Danish Hospital Discharge Register. Based on incidence rates from the general population the expected number of cases from this register was 716 women (737 observed) and 1217 men (1196 observed). The observed number did not differ significantly from the expected ( $P>0.10$ ).

Amount of alcohol intake was inversely associated with coronary heart disease among women and men (figure).

Among women, drinking on at least one day a week was associated with a lower risk of coronary heart disease than drinking more rarely than one day a week (table 2). Hazard ratios were similar for drinking on one day a week (0.64, 95% confidence interval 0.51 to 0.81), 2-4 days a week (0.63, 0.52 to 0.77), five or six days a week (0.79, 0.61 to 1.03), and seven days a week (0.65, 0.51 to 0.84). A test for trend not including women that were drinking more rarely than one day a week was statistically insignificant ( $P=0.49$ ).

Among men, drinking frequency was inversely associated with risk of coronary heart disease over the whole range of drinking frequencies (table 2). Hazard ratios were 0.93 (0.75 to 1.16) for drinking on one day a week, 0.78 (0.66 to 0.94) for 2-4 days a week, 0.71 (0.57 to 0.87) for five or six days a week, and 0.59 (0.48 to 0.71) for seven days a week ( $P$  for trend  $<0.0001$ ). The test for linear trend remained statistically significant after excluding men drinking more rarely than on one day a week ( $P<0.0001$ ).

A statistically significant interaction was found between sex and drinking frequency on the risk of coronary heart disease ( $P=0.02$ ).

Table 3 lists the hazard ratios of coronary heart disease for different combinations of alcohol amount and drinking frequency. Within similar categories of drinking frequency, women drinking the largest amounts generally had the lowest risk. For example, among women drinking on 2-4 days a week the hazard ratio was 0.78 (0.63 to 0.97) for 1-6 drinks a week, 0.74 (0.57 to 0.96) for 7-13 drinks a week, and 0.27 (0.13 to 0.58) for 14 or more drinks a week ( $P$  for trend  $<0.0001$ ). For men, hazard ratios were generally lowest for the most frequent intake within similar categories of amount (table 3). For example, among men drinking on average 7-13 drinks a week, hazard ratios of coronary heart disease were 0.89 (0.62 to 1.29) for drinking alcohol on one or less days a week, 0.81 (0.67 to 0.98) for 2-4 days a week, and 0.66 (0.52 to 0.83) for 5-7 days a week ( $P$  for trend = 0.0001). Within categories of drinking frequency, hazard ratios tended to be similar.

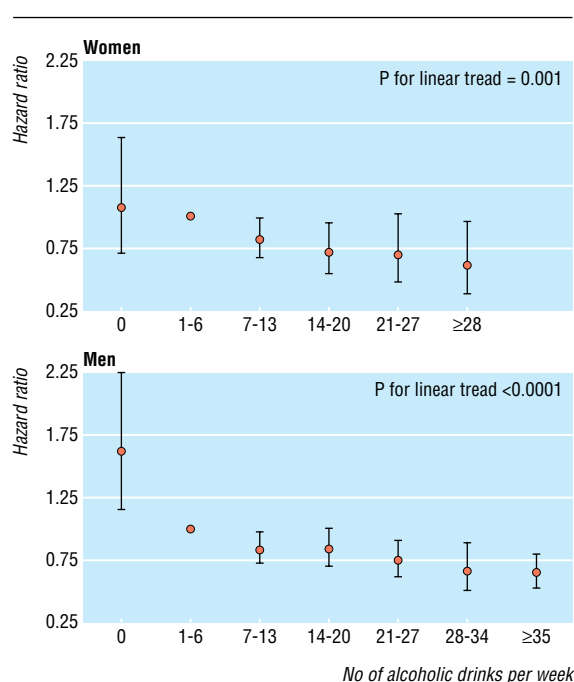
To examine the possibility that latent baseline symptoms of coronary heart disease such as angina pectoris might reduce the frequency of drinking alcohol, thereby biasing the results, we carried out analyses to compare the association between drinking frequency and coronary heart disease only including early cases—that is, cases that occurred within the first two years of follow-up ( $n=200$  women and  $n=381$  men)—with the association including only later cases ( $n=549$  women and  $n=902$  men). An inverse association was observed in both groups (data not shown).

**Table 1** Baseline characteristics of 28 448 women and 25 052 men participating in the Danish diet, cancer, and health study according-sex and frequency of drinking alcohol. Values are medians (5th-95th centiles) unless stated otherwise

Characteristic	Frequency of drinking alcohol (days/week)					
	Never	<1	1	2-4	5 or 6	7
<b>Women:</b>						
Number	613	7160	4320	9373	3117	3865
Median (range) age (years)	57 (50-64)	56 (50-64)	56 (50-64)	55 (50-63)	55 (50-64)	56 (50-64)
Alcohol intake (drinks/week)	0 (0)	1 (0.2-5)	3.5 (1.1-9)	6.6 (3-19)	12.7 (5.9-27)	19.1 (7.3-40)
No (%) current smokers	252 (41.1)	2556 (35.7)	1266 (29.3)	2531 (27.0)	963 (30.9)	1650 (42.7)
No (%) current heavy smokers*	78 (12.7)	508 (7.1)	251 (5.8)	600 (6.4)	256 (8.2)	502 (13.0)
No (%) educated at school ≤7 years	267 (43.6)	3072 (42.9)	1481 (34.3)	2456 (26.2)	598 (19.2)	877 (22.7)
Physical activity (hours/week)†	14 (4-41)	15 (5-40)	15 (5-38)	15 (6-35)	15 (6-34)	15 (5-38)
Body mass index	25.3 (19-38)	25.7 (20-36)	25.3 (20-34)	24.6 (20-32)	24.1 (20-32)	23.8 (19-32)
Vegetable intake (g/day)	146 (26-445)	157 (44-403)	169 (50-398)	178 (59-380)	186 (65-389)	172 (51-378)
Fruit intake (g/day)	188 (18-644)	207 (36-609)	213 (42-565)	202 (44-547)	195 (38-531)	167 (27-492)
Fish intake (g/day)	30 (3-84)	32 (9-85)	36 (11-85)	37 (13-83)	37 (13-83)	37 (11-86)
Saturated fat (% of total energy)	13 (8-19)	13 (8-18)	13 (8-17)	12 (8-17)	12 (8-16)	12 (8-16)
<b>Men:</b>						
Number	390	2464	2282	8718	4300	6898
Median (range) age (years)	56 (50-64)	56 (50-64)	56 (50-64)	55 (50-63)	55 (50-63)	56 (50-64)
Alcohol intake (drinks/week)	0 (0-0)	1.6 (0.3-8)	4.5 (1.3-13)	8.9 (3.7-25)	18.7 (7-42)	26.3 (9.5-60)
No (%) current smokers	192 (49.2)	1089 (44.2)	806 (35.3)	2973 (34.1)	1518 (35.3)	3249 (47.1)
No (%) current heavy smokers*	171 (43.8)	690 (28.0)	534 (23.4)	2171 (24.9)	1204 (28.0)	2407 (34.9)
No (%) educated at school ≤7 years	154 (39.5)	1175 (47.7)	895 (39.2)	2851 (32.7)	1213 (28.2)	2228 (32.3)
Physical activity (hours/week)†	12 (2-39)	11 (3-33)	11 (3-30)	11 (4-29)	11 (4-29)	11 (4-31)
Body mass index	25.9 (21-33)	26.5 (22-34)	26.3 (21-33)	26.1 (22-33)	26.1 (22-32)	26.0 (21-33)
Vegetable intake (g/day)	136 (25-393)	130 (38-335)	145 (47-343)	157 (53-341)	162 (55-340)	149 (44-336)
Fruit intake (g/day)	149 (14-589)	148 (20-519)	154 (28-505)	149 (27-471)	139 (24-436)	121 (16-415)
Fish intake (g/day)	34 (2-106)	36 (7-98)	39 (11-96)	42 (14-96)	43 (14-97)	43 (13-100)
Saturated fat (% of total energy)	15 (9-19)	14 (9-18)	14 (9-18)	13 (9-17)	13 (9-16)	12 (8-16)

\*Smoking more than 25 g of tobacco daily.

†Sum of recreational and household activities.



Hazard ratios (95% confidence intervals), adjusted for age, smoking, education, physical activity, body mass index, and total intake of fruit, vegetables, fish, and saturated fat, for coronary heart disease according to alcohol intake among women and men. Abstainers were not included in analyses for trend

## Discussion

The frequency of drinking alcohol is inversely associated with risk of coronary heart disease among men and this was independent of alcohol intake. Among women, alcohol intake and not drinking frequency was inversely associated with coronary heart disease.

A limitation of our study is that only 35% of the invited people participated and hence caution should be taken when generalising our findings. People who choose to participate may have a different risk profile and be in better health than those who decline. However, the observed incidence of coronary heart disease did not differ from that of the general population.

We found that the association between drinking frequency and coronary heart disease was different for men and women. The number of cases was substantially lower among women than among men, however, and hence results for women are less certain and warrant further study.

We cannot exclude the possibility that participants with early symptoms of coronary heart disease at baseline had reduced their drinking frequency, explaining the inverse association. However, this association persisted when we analysed early cases separately, indicating that the observed association is unlikely to be explained by this possible bias.

Some unhealthy traits (smoking and a low intake of fruit and vegetables) were common at both extremes of drinking frequency. Everyday drinking may be associated with borderline addictive behaviour, and a strong association between smoking



**Table 2** Hazard ratios (95% confidence intervals) of coronary heart disease according to drinking frequency among women and men

Variable	Frequency of drinking alcohol (days/week)						P for trend*
	Never	<1	1	2-4	5 or 6	7	
Women:							
No of cases	24	276	95	187	77	90	
Adjusted for age	1.01 (0.66 to 1.53)	1.00	0.60 (0.47 to 0.76)	0.56 (0.47 to 0.68)	0.69 (0.54 to 0.89)	0.62 (0.49 to 0.79)	0.0004
Adjusted for multiple factors†	0.92 (0.61 to 1.41)	1.00	0.64 (0.51 to 0.81)	0.63 (0.52 to 0.77)	0.79 (0.61 to 1.03)	0.65 (0.51 to 0.84)	0.007‡
Men:							
No of cases	39	180	140	424	195	305	
Adjusted for age	1.38 (0.98 to 1.95)	1.00	0.86 (0.69 to 1.07)	0.69 (0.58 to 0.83)	0.65 (0.53 to 0.79)	0.60 (0.50 to 0.73)	<0.0001
Adjusted for multiple factors†	1.44 (1.02 to 2.04)	1.00	0.93 (0.75 to 1.16)	0.78 (0.66 to 0.94)	0.71 (0.57 to 0.87)	0.59 (0.48 to 0.71)	<0.0001

\*Never drinkers not included in analyses for trend.  
†Age, smoking, education, physical activity, body mass index, total intake of fruit, vegetables, fish, and saturated fat.  
‡P for trend was 0.49 when women were excluded who never drink or drink on less than one day a week.

and drinking has been observed in many studies.<sup>15</sup> For the most rare drinkers, the unhealthy lifestyle may be explained by the fact that they were the poorest educated, which probably correlates with low social status. Also this category may include former alcoholics. Together, results for the extremes of drinking frequency are more likely to be residually confounded than results for the in between drinking frequencies and should be interpreted with caution. However, at least among men, we found an inverse association between drinking frequency and coronary heart disease over the entire range of drinking frequencies.

Drinking patterns in our study were constructed by combining information on average intake with drinking frequency, as done in another study.<sup>6</sup> We have avoided the term “binge drinking,” which is mostly defined as drinking a minimum number of drinks per occasion and we cannot comment on this with the present data.

Several explanations may account for a possible interaction between sex and drinking frequency. One explanation is sex specific drinking habits, such as drinking with meals. We cannot exclude that men who drink frequently are more likely to drink with meals, which may contribute to a greater risk reduction compared with men with a less frequent alcohol intake. The beneficial effect of meal related alcohol intake is, however, controversial.<sup>6</sup> It is unlikely that wine drinking, which may be more beneficial than drinking beer or spirits,<sup>16</sup> is responsible for our results because it has been shown that wine drinkers in this cohort drink less often than beer drinkers.<sup>17</sup> Differences in alcohol pharmacokinetics between sexes may be another explanation.<sup>7</sup>

The association between alcohol and coronary heart disease among women may be modified by menopausal status. Oestrogens have beneficial effects on the cardiovascular system, protecting women until menopause, when the incidence rapidly approaches that among men.<sup>18</sup> Moderate alcohol drinking is thought to increase oestrogen levels.<sup>8</sup> Few women in this study (17%) were premenopausal and our findings may be limited to postmenopausal women.

The inverse association between alcohol and coronary heart disease can be explained by several biologically plausible mechanisms, including dose dependent effects on high density lipoprotein levels, lower plasma fibrinogen levels, and reduced platelet aggregation.<sup>19</sup> These potential beneficial effects of alcohol must be considered along with potential adverse effects of a high intake, such as high blood pressure and increased triglyceride levels.<sup>20</sup> The question is if the balance between beneficial and harmful effects is affected by drinking pattern. Heavy weekend drinkers have been found to have a higher daily blood pressure<sup>21</sup> and to have greater between day variability in blood pressure than heavy daily drinkers.<sup>22, 23</sup> Results are conflicting as to whether drinking pattern modifies lipid levels. Some studies found that only regular drinking can raise high density lipoprotein levels,<sup>24, 25</sup> whereas others found this among weekend drinkers.<sup>26</sup> The presumed lowering effect of alcohol on fibrinogen levels has been found to be independent of drinking pattern (daily versus weekend drinking).<sup>27</sup> It has not been investigated if drinking pattern affects the presumed association between alcohol and increased oestrogen levels among women.

**Table 3** Hazard ratios (95% confidence intervals) of coronary heart disease according to drinking frequency and amount of alcohol intake among women and men

Alcohol intake (drinks/week)	Frequency of drinking alcohol (days/week)				P for trend
	Never	≤1	2-4	5-7	
Women:					
0	1.03 (0.68 to 1.56) (n=24)	—	—	—	—
1-6	—	1.00 (n=360)	0.78 (0.63 to 0.97) (n=114)	1.32 (0.84 to 2.07) (n=20)	0.57
7-13	—	0.67 (0.35 to 1.31) (n=9)	0.74 (0.57 to 0.96) (n=66)	0.82 (0.61 to 1.10) (n=52)	0.12
≥14	—	0.65 (0.16 to 2.61) (n=2)	0.27 (0.13 to 0.58) (n=7)	0.72 (0.57 to 0.92) (n=95)	0.01
P for trend	—	0.002	<0.0001	0.0003	—
Men:					
0	1.47 (1.05 to 2.06) (n=39)	—	—	—	—
1-6	—	1.00 (n=278)	0.80 (0.65 to 0.98) (n=141)	0.70 (0.41 to 1.17) (n=15)	0.02
7-13	—	0.89 (0.62 to 1.29) (n=31)	0.81 (0.67 to 0.98) (n=190)	0.66 (0.52 to 0.83) (n=90)	0.0001
14-20	—	1.10 (0.54 to 2.23) (n=8)	0.91 (0.68 to 1.23) (n=52)	0.68 (0.54 to 0.87) (n=90)	0.001
≥21	—	1.00 (0.32 to 3.13) (n=3)	0.67 (0.48 to 0.93) (n=41)	0.63 (0.53 to 0.74) (n=305)	<0.0001
P for trend	—	0.25	0.22	<0.0001	—

Hazard ratios are adjusted for age, education, smoking, physical activity, body mass index, and total intake of vegetables, fruit, fish, and saturated fat. Number of cases in parentheses.

Heavy alcohol drinking is positively associated with many problems such as liver diseases, cancers, and road crashes, and overall mortality is higher among individuals with a high alcohol intake compared with light consumers, reflecting that the beneficial effects of alcohol on coronary heart disease is by far exceeded by the detrimental effects of alcohol at these levels. Also, the beneficial effect of alcohol is probably confined to middle aged or older people.<sup>28</sup> Therefore the inverse association between alcohol intake and coronary heart disease should be viewed in this context when giving public health advice. In conclusion, we found that drinking frequency seemed to be the main determinant of the inverse association between alcohol intake and coronary heart disease among men, which confirms results from another study.<sup>6</sup> For women, amount of alcohol may be more important than frequency.

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**Contributors:** JT contributed to the conception and design of the study, the analysis and interpretation of data, and wrote the manuscript. MKJ, KJM, and MG contributed to the conception and design of the study, interpretation of data, and to critically revising the paper. AT and KO contributed to the design of the study, the acquisition of data, interpretation of data, and critically revising the paper. All authors approved the final version of the article. Katja Boll prepared the data file and Søren Rasmussen calculated incidence rates of coronary heart disease in the general Danish population. MG is the guarantor.

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- 1 Flesch M, Rosenkranz S, Erdmann E, Böhm M. Alcohol and the risk of myocardial infarction. *Basic Res Cardiol* 2001;96:128-35.
- 2 Maljutina S, Bobak M, Kurilovitch S, Gafarov V, Simonova G, Nikitin Y, et al. Relation between heavy and binge drinking and all-cause and cardiovascular mortality in Novosibirsk, Russia: a prospective cohort study. *Lancet* 2002;360:1448-54.
- 3 McElduff P, Dobson AJ. How much alcohol and how often? Population based case-control study of alcohol consumption and risk of a major coronary event. *BMJ* 1997;314:1159-64.
- 4 Trevisan M, Dorn J, Falkner K, Russell M, Ram M, Muti P, et al. Drinking pattern and risk of non-fatal myocardial infarction: a population-based case-control study. *Addiction* 2004;99:313-22.

- 5 Murray RP, Connett JE, Tyas SL, Bond R, Ekuma O, Silversides CK, et al. Alcohol volume, drinking pattern, and cardiovascular disease morbidity and mortality: is there a U-shaped function? *Am J Epidemiol* 2002;155:242-8.
- 6 Mukamal KJ, Conigrave KM, Mittleman MA, Camargo CA Jr, Stampfer MJ, Willett WC, et al. Roles of drinking pattern and type of alcohol consumed in coronary heart disease in men. *N Engl J Med* 2003;348:109-18.
- 7 Mumenthaler MS, Taylor JL, O'Hara R, Yesavage JA. Gender differences in moderate drinking effects. *Alcohol Res Health* 1999;23:55-64.
- 8 Register TC, Cline JM, Shively CA. Health issues in postmenopausal women who drink. *Alcohol Res Health* 2002;26:299-307.
- 9 Tjønneland A, Grønbaek M, Stripp C, Overvad K. Wine intake and diet in a random sample of 48 763 Danish men and women. *Am J Clin Nutr* 1999;69:49-54.
- 10 Overvad K, Tjønneland A, Haraldsdóttir J, Ewertz M, Jensen OM. Development of a semiquantitative food frequency questionnaire to assess food, energy and nutrient intake in Denmark. *Int J Epidemiol* 1991;20:900-5.
- 11 Tjønneland A, Overvad K, Haraldsdóttir J, Bang S, Ewertz M, Jensen OM. Validation of a semiquantitative food frequency questionnaire developed in Denmark. *Int J Epidemiol* 1991;20:906-12.
- 12 Jørgensen HJ, Frølund C, Gustafsen J, Mosbech H, Guldhammer B, Mosbech J. Registration of diagnoses in the Danish National Registry of patients. *Methods Inform Med* 1986;25:158-64.
- 13 Juel K, Helweg-Larsen K. The Danish registers of causes of death. *Dan Med Bull* 1999;46:354-7.
- 14 Shaper AG. Mortality and alcohol consumption. Non-drinkers shouldn't be used as baseline. *BMJ* 1995;310:325.
- 15 Bien TH, Burge R. Smoking and drinking: a review of the literature. *Int J Addict* 1990;25:1429-54.
- 16 Grønbaek M, Becker U, Johansen D, Gottschau A, Schnohr P, Hein HO, et al. Type of alcohol consumed and mortality from all causes, coronary heart disease, and cancer. *Ann Intern Med* 2000;133:411-9.
- 17 Grønbaek M, Tjønneland A, Johansen D, Stripp C, Overvad K. Type of alcohol and drinking pattern in 56,970 Danish men and women. *Eur J Clin Nutr* 2000;54:174-6.
- 18 Colditz GA, Willett WC, Stampfer MJ, Rosner B, Speizer FE, Hennekens CH. Menopause and the risk of coronary heart disease in women. *N Engl J Med* 1987;316:1105-10.
- 19 Agarwal DP. Cardioprotective effects of light-moderate consumption of alcohol: a review of putative mechanisms. *Alcohol Alcohol* 2002;37:409-15.
- 20 Puddey IB, Rakic V, Dimmitt SB, Beilin LJ. Influence of pattern of drinking on cardiovascular disease and cardiovascular risk factors—a review. *Addiction* 1999;94:649-63.
- 21 Wannamethee G, Shaper AG. Alcohol intake and variations in blood pressure by day of examination. *J Hum Hypertens* 1991;5:59-67.
- 22 Marmot MG, Elliott P, Shipley MJ, Dyer AR, Ueshima H, Beevers DG, et al. Alcohol and blood pressure: the INTERSALT study. *BMJ* 1994;308:1263-7.
- 23 Rakic V, Puddey IB, Burke V, Dimmitt SB, Beilin LJ. Influence of pattern of alcohol intake on blood pressure in regular drinkers: a controlled trial. *J Hypertens* 1998;16:165-74.
- 24 Gruchow HW, Hoffmann RG, Anderson AJ, Barboriak JJ. Effects of drinking patterns on the relationship between alcohol and coronary occlusion. *Atherosclerosis* 1982;43:393-404.
- 25 Hojnacki JL, Deschenes RN, Cluette-Brown JE, Mulligan JJ, Osmolski TV, Rencricca NJ, et al. Effect of drinking pattern on plasma lipoproteins and body weight. *Atherosclerosis* 1991;88:49-59.
- 26 Rakic V, Puddey IB, Dimmitt SB, Burke V, Beilin LJ. A controlled trial of the effects of pattern of alcohol intake on serum lipid levels in regular drinkers. *Atherosclerosis* 1998;137:243-52.
- 27 Dimmitt SB, Rakic V, Puddey IB, Baker R, Oosttryck R, Adams MJ, et al. The effects of alcohol on coagulation and fibrinolytic factors: a controlled trial. *Blood Coagul Fibrinolysis* 1998;9:39-45.
- 28 Fuchs CS, Stampfer MJ, Colditz GA, Giovannucci EL, Manson JE, Kawachi I, et al. Alcohol consumption and mortality among women. *N Engl J Med* 1995;332:1245-50.

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## What is already known on this topic

Alcohol intake is inversely associated with risk of coronary heart disease

In men, for the same weekly amount of alcohol intake, frequent drinkers have a lower risk of coronary heart disease than less frequent drinkers

Little is known about drinking pattern and the risk of coronary heart disease among women

## What this study adds

Intake may be more important than frequency for the inverse association between alcohol drinking and risk of coronary heart disease among women

In men, frequency is more important than alcohol intake



## PAPER

# The relation between drinking pattern and body mass index and waist and hip circumference

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**OBJECTIVES:** To study the association between alcohol drinking pattern and obesity.

**DESIGN:** Cross-sectional population study with assessment of quantity and frequency of alcohol intake, waist and hip circumference, height, weight, and lifestyle factors including diet.

**SUBJECTS:** In all, 25 325 men and 24 552 women aged 50–65 y from the Diet, Cancer and Health Study, Denmark, 1993–1997 participated in the study.

**MEASUREMENTS:** Drinking frequency, total alcohol intake, body mass index (BMI), and waist and hip circumference.

**RESULTS:** Among men, total alcohol intake was positively associated with high BMI ( $\geq 30$  kg/m<sup>2</sup>), large waist circumference ( $\geq 102$  cm) and inversely associated with small hip circumference ( $< 100$  cm). Among women, the total alcohol was associated with high BMI, large waist ( $\geq 88$  cm), and small hips only for the highest intake (28+ drinks/week). The most frequent drinkers had the lowest odds ratios (OR) for being obese. Among men, OR for having a high BMI were 1.39 (95% confidence interval: 1.36–1.64), 1.17 (1.02–1.34), 1.00 (reference), 0.87 (0.77–0.98), and 0.73 (0.65–0.82) for drinking 1–3 days/month, 1 day/week, 2–4 days/week, 5–6 days/week, and 7 days/week, respectively. Similar estimates were found for waist circumference. Corresponding results were found for women.

**CONCLUSION:** For a given level of total alcohol intake, obesity was inversely associated with drinking frequency, whereas the amount of alcohol intake was positively associated with obesity. These results indicate that frequent drinking of small amounts of alcohol is the optimal drinking pattern in this relation.

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**Keywords:** body mass index; waist circumference and hip circumference; alcohol; drinking pattern

## Introduction

The association between alcohol intake and body weight has been investigated in several studies. Alcoholic beverages are energy dense and are probably not substituting food but rather added to the total daily energy intake.<sup>1</sup> Also, inhibition of fat oxidation might occur as a consequence of the antilipolytic properties of metabolites from alcohol degradation.<sup>2</sup> These features could potentially promote fat storage and hence the risk of developing obesity. However, results from studies on alcohol intake and body weight are not conclusive.<sup>3–11</sup>

Pattern of alcohol drinking has been shown to be independently associated with the risk of coronary heart disease; a frequent intake of small amounts of alcohol seems to be more beneficial than a binge-like intake of larger amounts per drinking occasion.<sup>12–16</sup> It is not clear as to which mechanisms underlie this relation, but since obesity is an important risk factor for coronary heart disease, an association between drinking pattern and obesity could be explaining part of the association between drinking pattern and coronary heart disease. Little attention has been paid to the association between drinking pattern and obesity, but two smaller studies have suggested that, for the same total intake of alcohol, daily drinkers are leaner than nondaily drinkers.<sup>8,17</sup>

There has been some debate as to which is the best epidemiological measure of obesity. The World Health Organization has proposed the body mass index (BMI, kg/m<sup>2</sup>) and waist circumference,<sup>18</sup> but these measures may be too

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crude to use on their own. As an alternative, the waist-hip ratio has been proposed because it is a stronger predictor of morbidity and mortality,<sup>19</sup> probably because waist circumference is positively associated with adverse health outcomes, whereas large hips seem to have a protective influence.<sup>20,21</sup> However, the ratio may not always convey sufficient information<sup>22</sup> and interpretations are difficult due to different biological mechanisms: waist circumference reflects intra-abdominal fat, whereas hip circumference reflects different aspects of body composition in the gluteofemoral region (muscle mass, bone and fat mass).<sup>23</sup> Moreover, a ratio cannot express nonlinear relationships between hip and waist, which also may complicate interpretation of this measure.<sup>24</sup> Instead, using separate measures of waist and hip circumference, relative to body size, has been suggested.<sup>20,21</sup>

The aim of the present study was to examine the association between alcohol-drinking frequency and obesity. As measure of general obesity, we studied BMI, and as measures for fat distribution, we studied waist and hip circumference adjusted for BMI.

## Methods

During December 1993 to May 1997, 160 725 Danish men and women aged 50–65 y were invited by mail to participate in the population-based study 'Diet, Cancer and Health'.<sup>25</sup> Eligible subjects were born in Denmark and had no previous cancers at the time of inclusion. With the invitation, a detailed 192-item food frequency questionnaire including questions concerning total alcohol intake was enclosed. A visit at the study clinic was appointed by telephone with subjects who agreed to participate (27 178 men and 29 875 women (35%)). The food frequency questionnaire was scanned and interviewer checked during the clinic visit, where another questionnaire concerning lifestyle and background factors including information on frequency of alcohol intake was filled out.<sup>25</sup> The median time between administrations of the two questionnaires was 22 days, but for most participants the time between answering the questionnaires was probably shorter, since many chose to fill out the mail questionnaire in the waiting time during the clinic visit. A description of the development and validation of the food frequency questionnaire has been published previously.<sup>26,27</sup> The protocol was approved by the Ethical Committee (KF 01-116/96).

## Drinking frequency

In the background questionnaire, participants were asked to report their usual frequency of alcohol intake in seven possible response categories: never drink alcohol, less than once per month, one to three times per month, once a week, two to four times per week, five to six times per week, and daily.

## Total alcohol intake

Questions on total alcohol intake were stated in the food frequency questionnaire, where participants were asked to state their average quantity (during the last year) of alcohol consumption as the intake of specific amounts of each beverage: light, normal, and fortified beer (in number of bottles); red, white, and fortified wine (in number of glasses); and spirits (in number of drinks). Based on ethanol content in the different beverage types, these categories were converted into number of standard drinks (12 g alcohol) per week and added to yield an average measure of total alcohol intake.

## Body mass index

The participants' height and weight were measured in light clothes and without shoes. BMI was calculated as weight (kg) divided by squared height (m). When BMI was more than or equal to 30 kg/m<sup>2</sup>, subjects were considered obese in accordance with guidelines from the World Health Organization.<sup>18</sup>

## Waist and hip circumference

The participants' waist circumference was measured at the narrowest point between the lower rib and iliac crest and recorded to the nearest half centimeter in the standing position, in light clothes and relaxed breathing. Hip circumference was measured over the widest part of the buttocks and was recorded to the nearest half centimeter. The waist measures were dichotomized at 88 cm (women) and 102 cm (men) in accordance with guidelines from the World Health Organization.<sup>18</sup> For hip circumference, there are currently no guidelines, but we chose to dichotomize at 100 cm. This cut-point was selected because all-cause mortality has previously been shown to be increased beneath and decreased above this point among both men and women in this study population.<sup>22</sup>

## Putative confounders

**Education.** In the lifestyle questionnaire, education was estimated from length of basic schooling as 7 y or less, 8–10 y, or 11 y and longer.

**Smoking habits.** Subjects reported if they were never-smokers, ex-smokers, or current-smokers. Current-smokers reported number of daily cigarettes, cheroots, cigars, and pipes. Assuming one cigarette to be equivalent to 1 g, one cheroot or one pipe to 3 g, and one cigar to 5 g of tobacco, participants were categorized into five groups according to smoking habits (never-smokers, ex-smokers, 1–14, 15–24 and more than 24 g/day).

**Physical activity.** Subjects reported if they were physically active during leisure time, including doing sports,

housework, gardening, taking walks, and bicycling. For each activity, a dichotomized variable was computed with the cut-point defined as performing or not performing the activity in question. Participants also reported physical activity at work (sedentary work, standing work, light physical work, heavy physical work, or unemployment).

**Diet.** In order to adjust for the confounding effects of diet habits among participants, indicators presumed to represent a healthy and balanced diet were chosen from the food frequency questionnaire.<sup>28</sup> The chosen indicators were fish, total vegetable, salad, and fruit intake. For each indicator, the intake was dichotomized as high or low. The cut-points were defined as close to the 10th percentile of the sex-specific distribution as possible (fish, once a month or less; vegetables, once a week or less (women) and twice a month or less (men); salad, once a month or less; and fruit, twice a week or less (women) and twice a month or less (men)). The participants also indicated which type of fat they preferred for cooking and two groups were formed: the participants who mostly used olive oil in one group and those who mostly used other types of fat for cooking in another group. Use of fat spread on bread was used as a measure of saturated fat intake since one-third of saturated fat intake in Denmark is consumed as spread on bread. Two groups were formed: users and nonusers of fat spread on bread.

**Total energy intake.** Total energy intake, including energy from alcohol, was calculated from the information from the diet questionnaire by means of the software program Food Calc using population-specific standardized recipes and sex-specific portion sizes.<sup>29</sup> Total energy intake was modeled as linear splines with knots set at gender-specific quartiles.<sup>30</sup>

**Statistical analysis.** Subjects with incomplete information on the alcohol variables were excluded ( $N=104$ ). Since the aim of this study was to analyze the association between drinking frequency and obesity, while taking into account drinking amount, we excluded subjects reporting the following irrelevant, impossible or very unlikely combinations of frequency and amount: (a) drinking less than one drink per week irrespective of frequency ( $N=6586$ ), (b) drinking seven or more drinks per week at a frequency of less than monthly ( $N=65$ ), and (c) drinking 21 or more drinks per week at a frequency of thrice monthly or less frequent ( $N=40$ ). Also, subjects with incomplete information on any of the potential confounders ( $N=381$ ) were excluded. In all, 49 877 persons were eligible for this study.

The associations between drinking frequency and high BMI, large waist circumference or small hip circumference were described by logistic regression analyses, defining high BMI as  $\geq 30$  kg/m<sup>2</sup>, large waist circumference as  $\geq 102$  cm (men) and  $\geq 88$  cm (women), and small waist circumference as  $< 100$  cm (men and women). All analyses were stratified by sex.

When examining the independent effects of the amount of alcohol, the intake was categorized into five groups (1–6, 7–13, 14–20, 21–27, and  $\geq 28$  drinks/week), and when adjusting for its confounding effect on drinking frequency, modeled as linear splines with knots at gender-specific quintiles.<sup>30</sup> The cut-points for the categories were made so that, for instance, the categories 1–6 includes 6.9 drinks. To reduce colinearity among the BMI, waist, and hip measurements, we computed residuals from linear regression of respective waist and hip circumference on BMI. The resulting residuals were used for adjustment and modeled as linear splines with knots set at gender-specific quintiles.<sup>30</sup> Also, the following covariates were included in the adjusted models: age (as a linear variable), education, smoking habits, dietary indicators, and physical activity. When analyzing the effect of drinking frequency, participants drinking 2–4 days/week were chosen as the reference category because this consisted the largest group and to avoid using either extreme of drinking frequency as reference.

Pearson's correlation coefficient was calculated to examine the magnitude of correlations. The estimated odds ratios (ORs) are presented with 95% confidence intervals (CI).

The SAS/STAT for Windows (the Genmod procedure) was used for statistical analyses.<sup>31</sup>

Effect modifications between drinking frequency and total alcohol intake were evaluated by a nested log-likelihood test: a model including frequency and total alcohol intake was compared with a model also containing the interaction terms.

## Results

Total alcohol intake was positively correlated with drinking frequency, so that the median number of drinks per week was higher among frequent drinkers than among less frequent drinkers (Tables 1 and 2). Among men and women, the rarest drinkers ( $< 1$  day/month) and the most frequent drinkers (7 days/week) were more often smokers and had a lower intake of fruit and vegetables compared with participants in the other drinking frequencies. Also, a larger fraction of the most rare drinkers had the lowest level of education compared with more frequent drinking individuals. BMI was correlated to waist circumference (Pearson's correlation coefficient = 0.87 (men) and 0.85 (women)) and to waist circumference (Pearson's correlation coefficient = 0.78 (men) and 0.84 (women)).

For men, the association between total alcohol intake and high BMI was nonsignificant up to 20 drinks/week. Among men drinking 21–27 drinks/week, the OR was 1.32 (95% CI: 1.13–1.53), and for men drinking 28 or more drinks weekly, the OR was 1.78 (95% CI: 1.54–2.07). Compared with women drinking 1–6 drinks/week, the OR was 0.88 (95% CI: 0.79–0.99) for drinking 7–13 drinks/week, 0.94 (95% CI: 0.79–1.12) for drinking 14–20 drinks/week, 0.91 (95% CI: 0.72–1.14) for drinking 21–27 drinks/week and 1.38 (95% CI: 1.08–1.76) for drinking 28 or more drinks/week (Table 3).

**Table 1** Men: characteristics of the participants on categories of drinking frequency

Characteristics	Drinking frequency					
	<1 day/month	1–3 days/month	1 day/week	2–4 days/week	5–6 days/week	7 days/week
Subjects (number)	183	1742	2375	9190	4525	7310
Alcohol intake (median drinks per week)	1.7	2.4	4.5	8.9	18.7	26.2
Age (mean years)	56	57	56	56	56	56
Smoking (% current)	57	43	36	34	35	47
Education (% lowest level) <sup>a</sup>	57	47	40	33	29	33
Physical activity (% sedentary) <sup>b</sup>	20	20	19	16	16	19
Fruit intake (% in lowest consumption group) <sup>c</sup>	11	7	6	5	7	12
Vegetable intake (% in lowest consumption group) <sup>c</sup>	11	12	10	7	8	10

<sup>a</sup>Lowest level is defined as  $\leq 7$  y of education. <sup>b</sup>Sedentary is defined being physically active (performing sport, walking, or bicycling) for  $\leq 2$  h/week. <sup>c</sup>Low consumption is defined as below the approximately 10th percentile of the distribution.

**Table 2** Women: characteristics of the participants on categories of drinking frequency

Characteristics	Drinking frequency					
	<1 day/month	1–3 days/month	1 day/week	2–4 days/week	5–6 days/week	7 days/week
Subjects (number)	364	3406	4180	9494	3163	3945
Alcohol intake (median drinks per week)	1.3	1.8	3.6	6.6	12.7	19.1
Age (mean years)	58	57	56	56	56	57
Smoking (% current)	49	34	29	27	31	43
Education (% lowest level) <sup>a</sup>	50	41	34	26	19	23
Physical activity (% sedentary) <sup>b</sup>	16	12	13	10	11	14
Fruit intake (% in lowest consumption group) <sup>c</sup>	12	11	11	10	12	19
Vegetable intake (% in lowest consumption group) <sup>c</sup>	23	21	17	15	13	17

<sup>a</sup>Lowest level is defined as  $\leq 7$  y of education. <sup>b</sup>Sedentary is defined being physically active (performing sport, walking, or bicycling) for  $\leq 2$  h/week. <sup>c</sup>Low consumption is defined as below the approximately 10th percentile of the distribution.

**Table 3** OR (95% CI) for having a BMI  $\geq 30$  kg/m<sup>2</sup> according to total alcohol intake

Alcohol intake/drinks per week	Men				Women			
	N	Crude <sup>a</sup>	Adjusted <sup>b</sup>	Adjusted <sup>c</sup>	N	Crude <sup>a</sup>	Adjusted <sup>b</sup>	Adjusted <sup>c</sup>
28+	4437	1.19 (1.08–1.32)	1.77 (1.54–2.05)	1.78 (1.54–2.07)	958	0.84 (0.69–1.03)	1.42 (1.11–1.80)	1.38 (1.08–1.76)
21–27	3543	0.84 (0.75–0.94)	1.19 (1.03–1.38)	1.32 (1.13–1.53)	1477	0.53 (0.44–0.65)	0.87 (0.69–1.08)	0.91 (0.72–1.14)
14–20	3240	0.78 (0.70–0.89)	1.06 (0.92–1.22)	1.09 (0.94–1.26)	2855	0.57 (0.50–0.66)	0.89 (0.75–1.06)	0.94 (0.79–1.12)
7–13	7037	0.80 (0.73–0.88)	0.98 (0.88–1.09)	1.04 (0.93–1.16)	5947	0.63 (0.57–0.69)	0.85 (0.76–0.96)	0.88 (0.79–0.99)
1–6	7068	1 (reference)	1 (reference)	1 (reference)	13 315	1 (reference)	1 (reference)	1 (reference)

<sup>a</sup>Adjusted for age. <sup>b</sup>Adjusted for age and drinking frequency. <sup>c</sup>Adjusted for age, drinking frequency, education, smoking, physical activity (in leisure time and at work), and diet indicators.

For both men and women, there was a significant inverse association between drinking frequency and high BMI, so that the more frequent drinkers had the lowest probability of being obese (Table 4). Hence, the OR for a high BMI among daily drinking men was 0.73 (95% CI: 0.65–0.82), among men drinking on 5–6 days of the week, 0.87 (95% CI: 0.77–0.98), 2–4 days of the week, 1.00 (reference), 1 day/week, 1.17 (95% CI: 1.02–1.34), 1–3 days/month, 1.39 (95% CI: 1.17–1.64), and <1/month, 1.27 (95% CI: 0.86–1.90). For women, similar results were found (Table 4).

For men, there seemed to be an overall positive association between the total alcohol intake and ORs for large waist

circumference (Table 5). For women, there was no association between the total alcohol intake and ORs for large waist circumference, except among heavy drinkers (28+ drinks/week, OR=1.69 (95% CI: 1.41–2.03)). As with BMI, drinking frequency was inversely associated with large waist circumference among both men and women (Table 6).

Compared with drinking 1–6 drinks/week, men drinking more than 7 drinks/week had lower OR for having a small hip circumference (Table 7). For women, the association between total alcohol intake and small hips were statistical insignificant up to 27 drinks per week. For drinking 28 or

**Table 4** OR (95% CI) for having a BMI  $\geq 30$  kg/m<sup>2</sup> according to drinking frequency

Drinking frequency	Men				Women			
	N	Crude <sup>a</sup>	Adjusted <sup>b</sup>	Adjusted <sup>c</sup>	N	Crude <sup>a</sup>	Adjusted <sup>b</sup>	Adjusted <sup>c</sup>
7 days/week	7310	1.01 (0.92–1.10)	0.73 (0.65–0.82)	0.73 (0.65–0.82)	3945	0.74 (0.65–0.84)	0.71 (0.6–0.83)	0.71 (0.60–0.84)
5–6 days/week	4525	0.97 (0.88–1.08)	0.84 (0.75–0.95)	0.87 (0.77–0.98)	3163	0.75 (0.65–0.86)	0.77 (0.66–0.90)	0.81 (0.69–0.94)
2–4 days/week	9190	1 (reference)	1 (reference)	1 (reference)	9494	1 (reference)	1 (reference)	1 (reference)
1 day/week	2375	1.19 (1.05–1.34)	1.24 (1.08–1.41)	1.17 (1.02–1.34)	4180	1.34 (1.21–1.50)	1.21 (1.07–1.37)	1.17 (1.03–1.32)
1–3 days/month	1742	1.46 (1.28–1.66)	1.54 (1.31–1.82)	1.39 (1.17–1.64)	3406	1.77 (1.59–1.97)	1.47 (1.28–1.70)	1.39 (1.20–1.60)
<1 day/month	183	1.41 (0.97–2.05)	1.51 (1.02–2.24)	1.27 (0.85–1.90)	364	2.19 (1.70–2.83)	1.74 (1.32–2.30)	1.60 (1.20–2.12)

<sup>a</sup>Adjusted for age. <sup>b</sup>Adjusted for age and total alcohol intake (as linear splines). <sup>c</sup>Adjusted for age, total alcohol intake (as linear splines), education, smoking, physical activity (in leisure time and at work), and diet indicators.

**Table 5** OR (95% CI) for having a waist circumference  $\geq 102$  cm (men) and  $\geq 88$  cm (women) according to total alcohol intake

Alcohol intake/drinks per week	Men				Women			
	N	Crude <sup>a</sup>	Adjusted <sup>b</sup>	Adjusted <sup>c</sup>	N	Crude <sup>a</sup>	Adjusted <sup>b</sup>	Adjusted <sup>c</sup>
28+	4437	1.37 (1.26–1.49)	1.82 (1.62–2.06)	1.80 (1.59–2.04)	958	1.23 (1.06–1.42)	1.82 (1.52–2.17)	1.69 (1.41–2.03)
21–27	3543	0.94 (0.85–1.03)	1.21 (1.08–1.37)	1.26 (1.12–1.43)	1477	0.79 (0.69–0.90)	1.12 (0.96–1.32)	1.12 (0.96–1.32)
14–20	3240	0.90 (0.81–0.99)	1.13 (1.00–1.27)	1.15 (1.02–1.29)	2855	0.75 (0.68–0.83)	1.04 (0.91–1.18)	1.05 (0.92–1.19)
7–13	7037	0.87 (0.81–0.94)	1.02 (0.93–1.12)	1.04 (0.95–1.14)	5947	0.74 (0.69–0.80)	0.93 (0.85–1.01)	0.93 (0.85–1.02)
1–6	7068	1 (reference)	1 (reference)	1 (reference)	13 315	1 (reference)	1 (reference)	1 (reference)

<sup>a</sup>Adjusted for age and BMI residuals. <sup>b</sup>Adjusted for age, BMI residuals, and drinking frequency. <sup>c</sup>Adjusted for age, BMI residuals, drinking frequency, education, smoking, physical activity (in leisure time and at work), and diet indicators.

**Table 6** OR (95% CI) for having a waist circumference  $\geq 102$  cm (men) and  $\geq 88$  cm (women) according to drinking frequency

Drinking frequency	Men				Women			
	N	Crude <sup>a</sup>	Adjusted <sup>b</sup>	Adjusted <sup>c</sup>	N	Crude <sup>a</sup>	Adjusted <sup>b</sup>	Adjusted <sup>c</sup>
7 days/week	7310	1.12 (1.04–1.2)	0.81 (0.74–0.89)	0.78 (0.70–0.85)	364	0.89 (0.81–0.97)	0.76 (0.67–0.85)	0.75 (0.66–0.84)
5–6 days/week	4525	1.04 (0.95–1.13)	0.89 (0.81–0.98)	0.90 (0.82–0.99)	3406	0.90 (0.81–1.00)	0.86 (0.77–0.96)	0.89 (0.79–0.99)
2–4 days/week	9190	1 (reference)	1 (reference)	1 (reference)	4180	1 (reference)	1 (reference)	1 (reference)
1 day/week	2375	1.14 (1.03–1.27)	1.20 (1.07–1.34)	1.15 (1.02–1.29)	9494	1.24 (1.14–1.35)	1.22 (1.11–1.35)	1.18 (1.07–1.30)
1–3 days/month	1742	1.35 (1.21–1.51)	1.39 (1.20–1.60)	1.28 (1.11–1.48)	3163	1.44 (1.32–1.58)	1.37 (1.22–1.54)	1.28 (1.14–1.44)
<1 day/month	183	1.35 (0.98–1.86)	1.37 (0.97–1.92)	1.18 (0.83–1.68)	3945	1.97 (1.57–2.46)	1.81 (1.42–2.30)	1.62 (1.27–2.07)

<sup>a</sup>Adjusted for age and BMI residuals. <sup>b</sup>Adjusted for age, BMI residuals, and total alcohol intake. <sup>c</sup>Adjusted for age, BMI residuals, total alcohol intake, education, smoking, physical activity (in leisure time and at work), and diet indicators.

**Table 7** OR (95% CI) for having a hip circumference  $< 100$  cm according to total alcohol intake

Alcohol intake/drinks per week	Men				Women			
	N	Crude <sup>a</sup>	Adjusted <sup>b</sup>	Adjusted <sup>c</sup>	N	Crude <sup>a</sup>	Adjusted <sup>b</sup>	Adjusted <sup>c</sup>
28+	4437	1.02 (0.95–1.10)	0.81 (0.73–0.90)	0.73 (0.65–0.81)	958	1.41 (1.23–1.61)	0.87 (0.74–1.02)	0.78 (0.66–0.92)
21–27	3543	1.07 (0.98–1.16)	0.88 (0.80–0.98)	0.85 (0.77–0.94)	1477	1.50 (1.34–1.67)	0.98 (0.86–1.12)	0.93 (0.81–1.06)
14–20	3240	1.11 (1.03–1.21)	0.96 (0.87–1.06)	0.93 (0.84–1.03)	2855	1.56 (1.43–1.69)	1.08 (0.97–1.20)	1.02 (0.92–1.14)
7–13	7037	1.01 (0.94–1.08)	0.93 (0.86–1.00)	0.92 (0.85–0.99)	5947	1.27 (1.20–1.36)	1.04 (0.97–1.12)	1.01 (0.94–1.09)
1–6	7068	1 (reference)	1 (reference)	1 (reference)	13 315	1 (reference)	1 (reference)	1 (reference)

<sup>a</sup>Adjusted for age and BMI residuals. <sup>b</sup>Adjusted for age, BMI residuals, and drinking frequency. <sup>c</sup>Adjusted for age, BMI residuals, drinking frequency, education, smoking, physical activity (in leisure time and at work), and diet indicators.

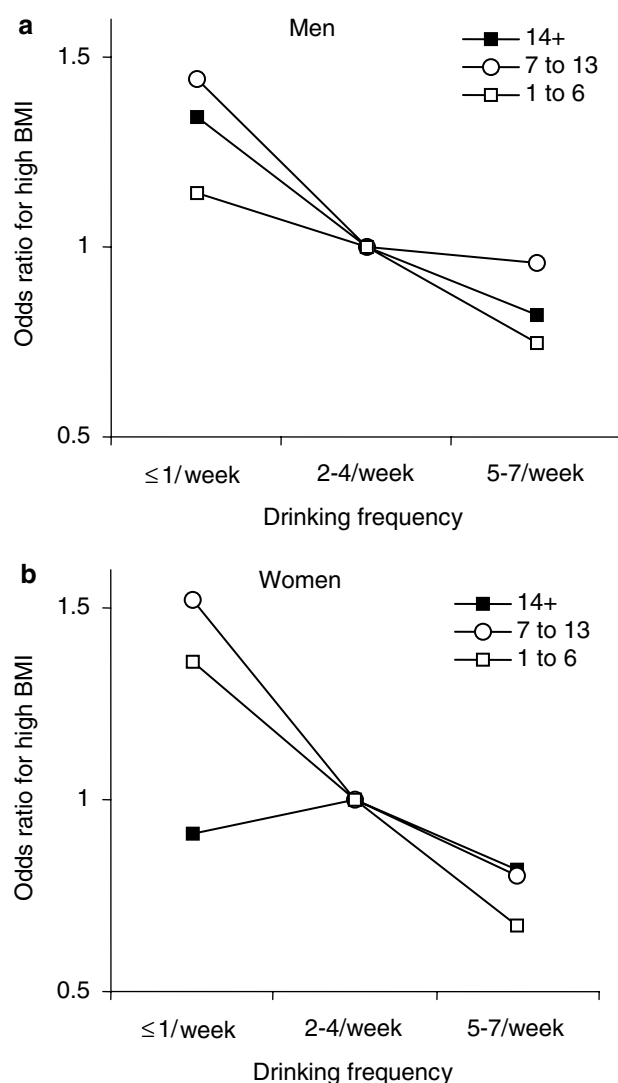
more drinks per week, the odds ratio for small hips was 0.78 (95% CI: 0.66–0.92) compared with drinking 1–6 drinks/week (Table 7). Drinking frequency was directly associated

with small hip circumference among both men and women, meaning that the more frequent drinkers had the highest probability of having small hips (Table 8).

**Table 8** OR (95% CI) for having a hip circumference <100 cm according to drinking frequency

Drinking frequency	Men				Women			
	N	Crude <sup>a</sup>	Adjusted <sup>b</sup>	Adjusted <sup>c</sup>	N	Crude <sup>a</sup>	Adjusted <sup>b</sup>	Adjusted <sup>c</sup>
7 days/week	7310	1.16 (1.09–1.23)	1.26 (1.16–1.37)	1.27 (1.17–1.38)	3945	1.56 (1.45–1.69)	1.56 (1.42–1.72)	1.50 (1.36–1.66)
5–6 days/week	4525	1.01 (0.94–1.09)	1.05 (0.97–1.14)	1.07 (0.99–1.16)	3163	1.23 (1.14–1.34)	1.21 (1.10–1.33)	1.18 (1.08–1.29)
2–4 days/week	9190	1 (reference)	1 (reference)	1 (reference)	9494	1 (reference)	1 (reference)	1 (reference)
1 day/week	2375	0.93 (0.85–1.02)	0.91 (0.82–1.00)	0.90 (0.81–1.00)	4180	0.79 (0.74–0.86)	0.82 (0.75–0.89)	0.81 (0.75–0.89)
1–3 days/month	1742	0.94 (0.85–1.04)	0.92 (0.81–1.04)	0.89 (0.78–1.01)	3406	0.82 (0.75–0.89)	0.87 (0.78–0.97)	0.85 (0.77–0.95)
<1 day/month	183	1.04 (0.78–1.40)	1.02 (0.75–1.40)	0.96 (0.70–1.31)	364	0.87 (0.70–1.08)	0.95 (0.75–1.19)	0.89 (0.70–1.13)

<sup>a</sup>Adjusted for age and BMI residuals. <sup>b</sup>Adjusted for age, BMI residuals, and total alcohol intake. <sup>c</sup>Adjusted for age, BMI residuals, total alcohol intake, education, smoking, physical activity (in leisure time and at work), and diet indicators.



**Figure 1** (a and b) Adjusted odds ratios (adjusted for age, education, smoking, physical activity (in leisure time and at work), and diet indicators) for having a BMI  $\geq 30$  kg/m<sup>2</sup> according to drinking frequency and strata of total alcohol intake with participants drinking 2–4/week as reference.

**Table 9** Men: number of participants in categories of drinking frequency and total alcohol intake

Drinking frequency	Total alcohol intake		
	1–6 drinks/week	7–13 drinks/week	14+ drinks/week
≤1 day/week	3631	531	138
2–4 days/week	3121	4189	1880
5–7 days/week	316	2317	9202

**Table 10** Women: number of participants in categories of drinking frequency and total alcohol intake

Drinking frequency	Total alcohol intake		
	1–6 drinks/week	7–13 drinks/week	14+ drinks/week
≤1 day/week	7478	390	82
2–4 days/week	5322	3310	862
5–7 days/week	515	2247	4346

Adjusting the alcohol ORs for drinking frequency (and *vice versa*) implied most of the effect of adjustment; inclusion of total energy intake (data not shown) and the other potential confounders in the model had little influence on size and precision of the estimates (Tables 3–8).

The inverse association between drinking frequency and large BMI (Figure 1a and b) was generally stable for different levels of total alcohol intake. The number of participants in categories of drinking frequency and total alcohol intake are shown in Table 9 (men) and Table 10 (women).

There were no significant interactions between total alcohol intake and drinking frequency for either high BMI ( $P > 0.2$  women,  $P > 0.2$  men), large waist circumference ( $P > 0.2$  women,  $P > 0.2$ men), or small hip circumference ( $P > 0.2$  women,  $P > 0.2$ men).

## Discussion

In this cross-sectional study, drinking frequency was independently and inversely associated with high BMI, meaning

that the lowest odds of being obese was observed among the most frequent drinkers. The results were consistent for men and women and for different levels of total alcohol intake. Exploring other anthropometric measures, large waist circumference and small hip circumference, we found that drinking frequency was inversely associated with large waist and directly associated with small hips.

This study has some limitations. Owing to the cross-sectional design, it is not possible to determine the temporality of the observed associations between alcohol-drinking frequency and obesity. It cannot be excluded that being overweight may cause a different alcohol-drinking behavior than being lean. Another limitation is that the study participants may not represent the general Danish population since only 35% of the invited persons participated. According to age- and sex-specific death rates of the general Danish population, less than half of the expected numbers of deaths had occurred at the end of 1999.<sup>32</sup> This is probably due to exclusion of subjects with previously diagnosed cancers and to a 'healthy participants effect'. However, considering the large study population and the broad spectrum of drinking patterns there seems no reason to assume that the observed associations between drinking frequency and obesity cannot be extrapolated to nonparticipants. We have no information on alcoholism among the study participants and can hence not exclude that individuals with addictive behavior are affecting our results. However, because of the above-stated arguments, we do not expect the fraction of alcoholics in our study cohort to be larger than in the general population.

We chose to study both BMI and waist and hip circumference since these measures probably contain different information on different aspects of obesity. Hip and waist circumference have shown opposite associations with all-cause mortality as well as incidence of cardiovascular disease and coronary heart disease after adjustment for BMI, which is a rationale for combining these measures.<sup>20–22</sup>

Strengths of the present study include its size and ability to adjust for many potential confounders. We included age, total alcohol and energy intake, education, smoking, physical activity, and dietary factors as covariates in the analyses. Most of the effect of controlling the estimates for drinking frequency for these potential confounders was due to the adjustment for total alcohol intake. Adjustment for the other potential confounders had little influence on size and CIs of the estimates for drinking frequency. A possible residual confounder of our results was socioeconomic status, since earlier studies have shown a binge-like drinking pattern to be associated with negative social circumstances.<sup>33</sup> In the present study, adjustment was made for education, which is expected to correlate highly with social status. However, this affected estimates marginally only. More detailed information on other social factors was not accounted for.

We found that some unhealthy traits (smoking and a low fruit and vegetable intake) were most common at both

extremes of drinking frequency (<1 day/month and 7 days/week). Everyday drinking may be associated with borderline addictive behavior and a strong association between smoking and drinking has been observed in many studies.<sup>34</sup> In the other end, for the most rare drinkers, the unhealthy lifestyle may be explained by the fact that they were also the poorest educated, which probably correlates with low social status. Also, this category may include former alcoholics who used to belong to the category of everyday drinkers. All taken together, this could indicate that our results for the extremes of drinking frequency may be more likely to be residually confounded than results for the in-between drinking frequencies and therefore should be interpreted with greater caution.

Our results agree with another cross-sectional study that demonstrated an inverse association between frequency of alcohol consumption, reported as monthly (less than weekly), weekly and daily alcohol drinking, and abdominal obesity measured as sagittal diameter.<sup>17</sup> This finding as well as our finding need to be confirmed in a prospective setting in order to clarify the causality of the association.

Alcohol is degraded in the liver by alcoholdehydrogenase, and to some extent also by the microsomal ethanol-oxidizing system.<sup>35</sup> The latter is induced by chronic alcohol intake and is presumably of less importance for moderate blood concentrations of alcohol. However, it has been suggested that alcoholdehydrogenase and microsomal ethanol-oxidizing system in conjunction may constitute a futile cycle system, so that the energy from alcohol mostly results in increased thermogenesis. If, in fact, such a cycle is of any physiological significance, drinking frequency may be important for the degree of microsomal ethanol-oxidizing system activation, and hence for the level of energy from alcohol that can be used as energy. If a frequent drinking pattern is more effective in inducing microsomal ethanol-oxidizing system than a nonfrequent drinking pattern, this may be the biologic mechanism behind our results.<sup>36</sup> Also, low doses of alcohol may stimulate energy expenditure since alcohol has an acute thermogenic effect.<sup>37</sup> It is possible that this effect is influenced by drinking pattern, so that an increased thermogenesis induced by a frequent low intake of alcohol is more sufficient in outbalancing the additional energy from the alcohol than for a corresponding less frequent intake of higher amounts per drinking occasion.

In summary, we found significant inverse associations between alcohol-drinking frequency and obesity, so that for a given amount of total alcohol intake, the most frequent drinkers had the lowest odds ratio of being obese. The association was the same for men and women. Given the existing evidence implicating that alcohol-drinking pattern is important for the association between alcohol intake and risk of coronary heart disease, it seems likely that obesity may be explaining part of this association.

## Acknowledgements

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## References

- Colditz GA, Giovannucci E, Rimm EB, Stampfer MJ, Rosner B, Speizer FE, Gordis E, Willett WC. Alcohol intake in relation to diet and obesity in women and men. *Am J Clin Nutr* 1991; **54**: 49–55.
- Crouse JR, Gerson CD, DeCarli LM, Lieber CS. Role of acetate in the reduction of plasma free fatty acids produced by ethanol in man. *J Lipid Res* 1968; **9**: 509–512.
- Cigolini M, Targher G, Bergamo AI, Tonoli M, Filippi E, Muggeo M, De Sandre G. Moderate alcohol consumption and its relation to visceral fat and plasma androgens in healthy women. *Int J Obes Relat Metab Disord* 1996; **20**: 206–212.
- Dallongeville J, Marecaux N, Ducimetiere P, Ferrieres J, Arveiler D, Bingham A, Ruidavets JB, Simon C, Amouyel P. Influence of alcohol consumption and various beverages on waist girth and waist-to-hip ratio in a sample of French men and women. *Int J Obes Relat Metab Disord* 1998; **22**: 1178–1183.
- Haffner SM, Stern MP, Hazuda HP, Pugh J, Patterson JK, Malina R. Upper body and centralized adiposity in Mexican Americans and non-Hispanic whites: relationship to body mass index and other behavioral and demographic variables. *Int J Obes* 1986; **10**: 493–502.
- Han TS, Bijnen FC, Lean ME, Seidell JC. Separate associations of waist and hip circumference with lifestyle factors. *Int J Epidemiol* 1998; **27**: 422–430.
- Lahti-Koski M, Pietinen P, Heliovaara M, Vartiainen E. Associations of body mass index and obesity with physical activity, food choices, alcohol intake, and smoking in the 1982–1997 FINRISK Studies. *Am J Clin Nutr* 2002; **75**: 809–817.
- Mannisto S, Pietinen P, Haukka J, Ovaskainen ML, Albanes D, Virtamo J. Reported alcohol intake, diet and body mass index in male smokers. *Eur J Clin Nutr* 1996; **50**: 239–245.
- Marti B, Tuomilehto J, Salomaa V, Kartovaara L, Korhonen HJ, Pietinen P. Body fat distribution in the Finnish population: environmental determinants and predictive power for cardiovascular risk factor levels. *J Epidemiol Community Health* 1991; **45**: 131–137.
- Sakurai Y, Umeda T, Shintchi K, Honjo S, Wakabayashi K, Todoroki I, Nishikawa H, Ogawa S, Katsurada M. Relation of total and beverage-specific alcohol intake to body mass index and waist-to-hip ratio: a study of self-defense officials in Japan. *Eur J Epidemiol* 1997; **13**: 893–898.
- Laitinen J, Pietilainen K, Wadsworth M, Sovio U, Jarvelin MR. Predictors of abdominal obesity among 31-y-old men and women born in Northern Finland in 1966. *Eur J Clin Nutr* 2004; **58**: 180–190.
- McElduff P, Dobson AJ. How much alcohol and how often? Population based case-control study of alcohol consumption and risk of a major coronary event. *BMJ* 1997; **314**: 1159–1164.
- Mukamal KJ, Conigrave KM, Mittleman MA, Camargo Jr CA, Stampfer MJ, Willett WC, Rimm EB. Roles of drinking pattern and type of alcohol consumed in coronary heart disease in men. *N Engl J Med* 2003; **348**: 109–118.
- Murray RP, Connett JE, Tyas SL, Bond R, Ekuma O, Silversides CK, Barnes GE. Alcohol volume, drinking pattern, and cardiovascular disease morbidity and mortality: is there a U-shaped function? *Am J Epidemiol* 2002; **155**: 242–248.
- Laatikainen T, Manninen L, Poikolainen K, Vartiainen E. Increased mortality related to heavy alcohol intake pattern. *J Epidemiol Community Health* 2003; **57**: 379–384.
- Malyutina S, Bobak M, Kurilovitch S, Gafarov V, Simonova G, Nikitin Y, Marmot M. Relation between heavy and binge drinking and all-cause and cardiovascular mortality in Novosibirsk, Russia: a prospective cohort study. *Lancet* 2002; **360**: 1448–1454.
- Dorn JM, Hovey K, Muti P, Freudenheim JL, Russell M, Nochajski TH, Trevisan M. Alcohol drinking patterns differentially affect central adiposity as measured by abdominal height in women and men. *J Nutr* 2003; **133**: 2655–2662.
- Geneva: World Health Organization. Obesity: preventing and managing the global epidemic. *Report of a WHO Consultation*. Geneva, Switzerland, 894, WHO Technical Report Series, 2000.
- Welborn TA, Dhaliwal SS, Bennett SA. Waist-hip ratio is the dominant risk factor predicting cardiovascular death in Australia. *Med J Aust* 2003; **179**: 580–585.
- Lissner L, Bjorkelund C, Heitmann BL, Seidell JC, Bengtsson C. Larger hip circumference independently predicts health and longevity in a Swedish female cohort. *Obes Res* 2001; **9**: 644–646.
- Heitmann BL, Frederiksen P, Lissner L. Hip circumference and cardiovascular morbidity and mortality in men and women. *Obes Res* 2004; **12**: 482–487.
- Bigaard J, Frederiksen K, Tjonnelland A, Thomsen BL, Overvad K, Heitmann BL, Sorensen TI. Waist and hip circumferences and all-cause mortality: usefulness of the waist-to-hip ratio? *Int J Obes Relat Metab Disord* 2004; **28**: 741–747.
- Molarius A, Seidell JC. Selection of anthropometric indicators for classification of abdominal fatness—a critical review. *Int J Obes Relat Metab Disord* 1998; **22**: 719–727.
- Allison DB, Paultre F, Goran MI, Poehlman ET, Heymsfield SB. Statistical considerations regarding the use of ratios to adjust data. *Int J Obes Relat Metab Disord* 1995; **19**: 644–652.
- Tjonnelland A, Thomsen BL, Stripp C, Christensen J, Overvad K, Mellemeaer L, Gronbaek M, Olsen JH. Alcohol intake, drinking patterns and risk of postmenopausal breast cancer in Denmark: a prospective cohort study. *Cancer Causes Control* 2003; **14**: 277–284.
- Overvad K, Tjonnelland A, Haraldsdottir J, Ewertz M, Jensen OM. Development of a semiquantitative food frequency questionnaire to assess food, energy and nutrient intake in Denmark. *Int J Epidemiol* 1991; **20**: 900–905.
- Tjonnelland A, Overvad K, Haraldsdottir J, Bang S, Ewertz M, Jensen OM. Validation of a semiquantitative food frequency questionnaire developed in Denmark. *Int J Epidemiol* 1991; **20**: 906–912.
- Tjonnelland A, Gronbaek M, Stripp C, Overvad K. Wine intake and diet in a random sample of 48 763 Danish men and women. *Am J Clin Nutr* 1999; **69**: 49–54.
- Lauritsen J. *FoodCalc Release 1.3*. 1998. [www.foodcalc.dk](http://www.foodcalc.dk)
- Greenland S. Dose-response and trend analysis in epidemiology: alternatives to categorical analysis. *Epidemiology* 1995; **6**: 356–365.
- SAS Institute Inc. *SAS/STAT Software: The GENMOD Procedure, Release 8.02*. SAS Institute Inc.: Cary, NC; 1993.
- Tjonnelland AM, Overvad OK. Diet, cancer and health—a population study and establishment of a biological bank in Denmark. *Ugeskr Laeger* 2000; **162**: 350–354.
- Rehm J, Gmel G. Patterns of alcohol consumption and social consequences. Results from an 8-year follow-up study in Switzerland. *Addiction* 1999; **94**: 899–912.
- Bien TH, Burge R. Smoking and drinking: a review of the literature. *Int J Addict* 1990; **25**: 1429–1454.
- Lieber CS. Microsomal ethanol-oxidizing system (MEOS): the first 30 years (1968–1998)—a review. *Alcohol Clin Exp Res* 1999; **23**: 991–1007.
- Lands WE, Zakhari S. The case of the missing calories. *Am J Clin Nutr* 1991; **54**: 47–48.
- Buermann B, Astrup A. How does the body deal with energy from alcohol? *Nutrition* 2001; **17**: 638–641.



# Drinking pattern and mortality in middle-aged men and women

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## ABSTRACT

**Aims** To address the prospective association between alcohol drinking pattern and all-cause mortality.

**Design** Population-based cohort study conducted between 1993 and 2003.

**Setting** Denmark.

**Participants** A total of 26 909 men and 29 626 women aged 55–65 years.

**Measurements** We obtained risk estimates for all-cause mortality for different levels of quantity and frequency of alcohol intake adjusted for life-style factors, including diet.

**Findings** During follow-up, 1528 men and 915 women died. For the same average consumption of alcohol, a non-frequent intake implied a higher risk of death than a frequent one.

**Conclusions** Drinking pattern and not just the total amount of alcohol consumed is important for the association between alcohol intake and mortality. These results suggest that future public guidelines concerning sensible alcohol drinking should include messages about drinking pattern together with quantity of alcohol.

**KEYWORDS** Alcohol drinking, drinking behaviour, follow-up studies, mortality.

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## INTRODUCTION

A large number of prospective studies have consistently reported a J-shaped relation between an average measure of alcohol intake and all-cause mortality [1–3]. This characteristic form most probably reflects a beneficial effect on the cardiovascular system of light alcohol intake, and harmful implications, such as liver cirrhosis and cancer, of high consumption. These associations have been addressed mainly without taking drinking pattern into account, with the exception of some recent studies [4–10]. Although these studies differed with regard to type and quality of measures of drinking patterns, results implied consistently the importance of drinking pattern in addition to the total quantity consumed. Most recently it has been suggested that drinking frequency, and not

the total amount of alcohol, is the primary determinant of the inverse association between alcohol intake and coronary heart disease [8]. However, it seems unlikely that the cardioprotective benefits would outweigh the detrimental effects of a high alcohol intake, regardless of the drinking pattern. Hence, for public health purposes, a more universal outcome such as mortality from all-causes is relevant, because it constitutes a scientific basis for creating guidelines on sensible drinking.

The aim of the present study is to investigate the association between frequency of drinking episodes for a given level of total alcohol consumption and all-cause mortality. We use data from a large prospective cohort study consisting of middle-aged men and women and have the ability to adjust for related life-style factors such as diet and physical activity.

## METHODS

During December 1993 to May 1997, 160 725 Danish men and women aged 50–65 years were invited by mail to participate in the population-based study 'Diet, Cancer and Health' [11]. Eligible subjects were born in Denmark and had no previous cancers at the time of inclusion. With the invitation, a detailed 192-item food frequency questionnaire including questions concerning average alcohol intake was enclosed. A first visit to the study clinic was arranged by telephone with subjects who agreed to participate [27 178 men and 29 875 women (35%)]. The food frequency questionnaire was returned during the clinic visit, where another questionnaire concerning life-style and background factors including information on frequency of alcohol intake was completed. A description of the food frequency questionnaire has been published previously [12]. The study was conducted in accordance with the Helsinki Declaration II and was approved by the Ethical Committees for the Copenhagen and the Aarhus municipalities (KF 01–116/96).

### Alcohol intake and drinking patterns

In the background questionnaire, subjects reported their usual frequency of alcohol intake in seven possible response categories: never drink alcohol, less than once per month, one to three times per month, once a week, two to four times per week, five to six times per week and daily.

In the food frequency questionnaire, participants were asked to state their average quantity (during the last year) of alcohol consumption as the intake of specific amounts of each beverage: light, normal and strong beer (in number of bottles); red, white and fortified wine (in number of glasses); and spirits (in number of drinks). The possible response categories were no alcohol intake, less than one per month, one per month, two to three per month, one per week, two to four per week, five to six per week, one per day, two to three per day, four to five per day, six to seven per day and eight or more per day. Based on ethanol content in the different beverage types, these categories were converted into number of standard drinks (12 g alcohol) per week and added to yield an average measure of total alcohol intake.

For a given quantity of total alcohol intake, two groups of drinkers were formed to differentiate between individuals drinking little alcohol frequently and individuals consuming a larger quantity of alcohol more rarely. Frequent drinkers were defined as individuals who consumed alcohol at least 2 days per week and non-frequent drinkers were defined as subjects who used to drink alcohol less often. Abstainers were defined as subjects who, in both questionnaires, reported never to drink.

For women, total alcohol intake was categorized into five levels (none, less than one, one to six, seven to 13 and more than 13 drinks per week) and for men, total alcohol intake was categorized into six levels (none, less than one, one to six, seven to 13, 14–20 and more than 20 drinks per week).

### Education

In the life-style questionnaire, education was estimated from length of basic schooling as 7 years or less, 8–10 years or 11 years and longer.

### Smoking habits

Subjects reported if they were never-smokers, ex-smokers or current-smokers. Current smokers reported number of daily cigarettes, cheroots, cigars and pipes. Assuming one cigarette to be equivalent to 1 g, one cheroot or one pipe to 3 g and one cigar to 5 g tobacco, total amount of smoking was calculated. Two variables were constructed, one indicating smoking status (never, ex, current) and one indicating amount of smoking (0 for never and ex-smokers, and 1–14 g per day, 15–24 g per day or more than 24 g per day for current smokers).

### Body mass index

The participants' height and weight were measured in light clothes and without shoes. Body mass index (BMI) was calculated as weight (kg) divided by squared height (m) and modelled as linear splines after log-transformation with knots at 18.5, 25, and 30 kg/m<sup>2</sup>. These limits were set in accordance with guidelines from the World Health Organization [13].

### Physical activity

Subjects reported if they were physically active during leisure time, including undertaking sports, housework, gardening, taking walks and bicycling. For each activity, a dichotomized variable was computed with the cut-point defined as performing or not performing the activity in question.

### Diet

Indicators of a healthy diet among the participants were chosen from the food frequency questionnaire. For intake of fish, cooked vegetables, salad and fruit, respectively, the intake was dichotomized as high or low. The cut-points were defined as close as possible to the 10th percentile of the sex-specific distribution (fish, once a month or less; vegetables, twice per month or less; salad, once a month or less; and fruit, once a week or less). The participants also indicated which type of fat used mainly for cooking

and two groups were formed: the participants in one group who used mainly olive oil and those in another group who used mainly other types of fat for cooking. Use of fat spread on bread was used as a measure of saturated fat intake because one-third of saturated fat intake in Denmark is consumed as spread on bread. Two groups were formed, users and non-users of fat spread on bread.

### Diseases before baseline

Information on the participants' health status when entering the study cohort was obtained from the population-based Danish Patient Register, which keeps records of all somatic hospitalizations in Denmark since 1977. The diagnoses are classified according to the World Health Organization's *International Classification of Diseases*, 8th revision (ICD-8). By linking the study cohort to this register, information on the participants' health status from 1977 to baseline (1993–97) was obtained. Dichotomized variables were constructed for stroke (ICD-8 codes: 430–438), acute myocardial infarction (ICD-8 code: 410), angina pectoris (ICD-8 codes: 411 and 413), other cardiovascular diseases (ICD-8 codes: 390–409, 412, 414–429 and 439–458) and other diagnoses implying diseases with a chronic character. The latter includes infectious, endocrinological, nervous system, chronic lung, gastrointestinal, alcohol-related, urological and muscular diseases (ICD-8 codes: 40–46, 79–83, 93–95, 240–289, 340–358, 490–493, 530–537, 560–573, 577, 580–584 and 710–738).

### Follow-up

Vital status of the study population sample was followed until 20 February 2003 by using the unique person identification number in the Civil Registration System. The observation time for each participant was the period from enrolment into the study (December 1993 to May 1997) until 20 February 2003, death ( $n = 2443$ ), emigration ( $n = 255$ ) or disappearance ( $n = 4$ ), whichever came first.

### Statistical analysis

Subjects with incomplete information on alcohol intake ( $n = 104$ ) or on any of the potential confounders ( $n = 240$ ) were excluded from the analyses. A few subjects had reported conflicting answers between their average total alcohol intake and the frequency of alcohol intake, and as it was difficult to categorize such subjects they were excluded from the analyses ( $n = 174$ ). A total of 56 535 subjects were eligible for this study.

Pearson's correlation coefficient was calculated to examine the magnitude of correlation between drinking frequency and amount of drinking.

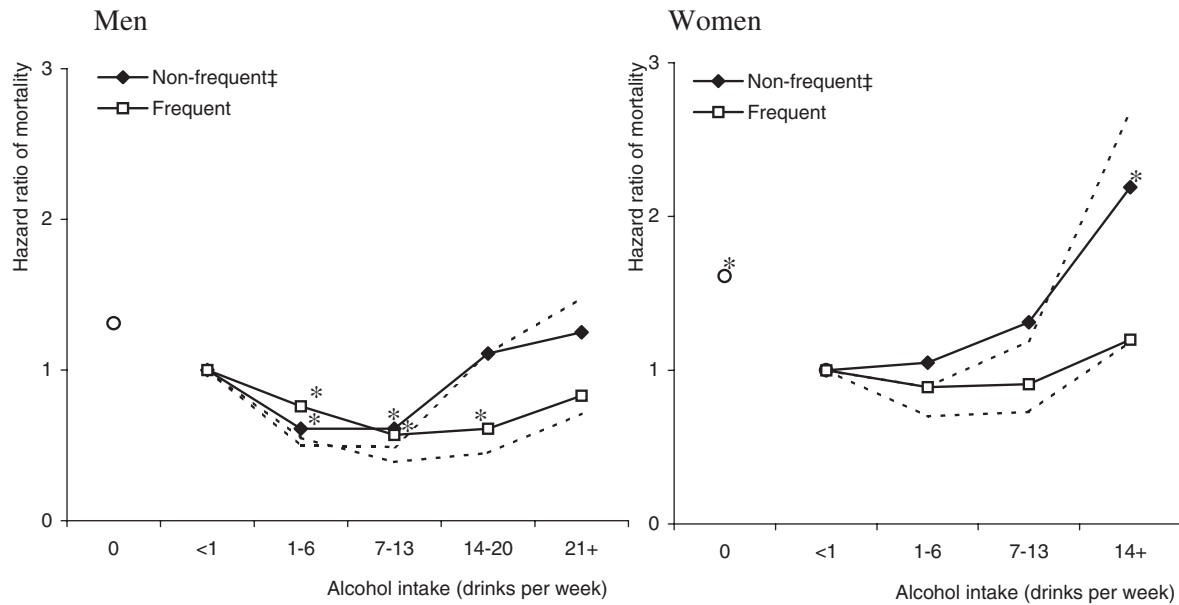
Risk estimates were computed by means of Cox proportional hazard regression models [14] (SAS/STAT program software). Age was used as the time axis to ensure that the estimation procedure was based on comparisons of individuals at the same age. The analyses were corrected for delayed entry, such that individuals were considered at risk only from the age at entry into the study cohort. In one model (Fig. 1), the frequency of drinking was categorized into two levels (subjects consuming alcohol at least 2 days per week and subjects consuming alcohol less often) for each level of total alcohol intake. In another model (Table 1a,b), the frequency of drinking was categorized into four levels (once per week or less, two to four times per week, five to six times per week and daily drinking) for every level of total alcohol intake. For each model, all combinations of frequency and level of total alcohol intake was entered simultaneously. Having had a diagnosis of a disease before baseline, school education, smoking, BMI, intake of fish, fruit, salad and vegetables, use of olive oil in cooking and of fat on bread were included as covariates in the adjusted model. All analyses were performed for each sex separately. The assumption of proportional hazards in the Cox model was tested for each covariate by evaluating the parallelism of the stratified survival curves graphically and by constructing time-dependent variables for the covariates in question and testing these for statistical significance. No violations were detected. Analyses were repeated after exclusion of subjects with a disease before baseline.

We used the Wald test to examine the joint hypothesis of differences in the hazard ratio for mortality between non-frequent and frequent drinkers for a weekly alcohol intake of more than one drink per week.

## RESULTS

Among men who reported to consume any alcohol, 21 083 did so at least twice per week while 4450 drank alcohol less frequently (Table 2a). Among alcohol-consuming women, 16 659 were frequent drinkers and 8103 were non-frequent drinkers (Table 2b). Among both men and women, the median alcohol consumption was higher among frequent drinkers for each category of total alcohol intake than among the corresponding non-frequent drinkers. Overall, drinking frequency was correlated moderately to amount of drinking [Pearson's correlation coefficient = 0.70 (women) and 0.63 (men)]. Among both men and women, non-frequent drinkers generally had a lower educational level, were more often smokers, more often obese and eating fewer vegetables and fruit than frequent drinkers.

During a mean follow-up of 6.8 years, 1528 men and 915 women died. The adjusted hazard ratios for non-frequent and frequent drinkers according to total alcohol



**Figure 1** Hazard ratios (age-adjusted estimates are represented by broken lines and fully adjusted<sup>†</sup> estimates by full lines) for all-cause mortality according to quantity and frequency of alcohol intake in men and women<sup>‡</sup> (frequent = at least 2 drinking days per week, non-frequent = less than 2 drinking days per week).

<sup>†</sup>Adjusted for education, smoking, BMI, physical activity, diet and diseases before baseline;

<sup>‡</sup>reference category is drinkers of less than one but more than zero drinks per week.

\*p = value less than 0.05 compared to reference category

**Table 1** Adjusted hazard ratios\* of all-cause mortality (95% confidence limits) according to quantity and frequency of alcohol intake.

Alcohol intake, drinks per week	Frequency of alcohol intake				
	Abstainers	Once per week or less	2–4 times per week	5–6 times per week	Daily
(a) Men					
0	1.31 (0.96–1.78)	—	—	—	—
Less than one	—	1.00 (reference)	—	—	—
1–6	—	0.61 (0.47–0.79)	0.74 (0.56–0.97)	0.91 (0.50–1.66)	0.84 (0.39–1.82)
7–13	—	0.61 (0.42–0.90)	0.56 (0.43–0.73)	0.51 (0.36–0.73)	0.63 (0.44–0.89)
14–20	—	1.11 (0.62–2.00)	0.61 (0.43–0.87)	0.52 (0.35–0.76)	0.67 (0.49–0.93)
21 +	—	1.25 (0.70–2.24)	1.03 (0.76–1.41)	0.68 (0.51–0.92)	0.84 (0.66–1.06)
(b) Women					
0	1.62 (1.19–2.19)	—	—	—	—
Less than one	—	1.00 (reference)	—	—	—
1–6	—	1.05 (0.85–1.30)	0.90 (0.70–1.17)	0.72 (0.32–1.64)	0.83 (0.31–2.24)
7–13	—	1.30 (0.85–2.01)	0.94 (0.72–1.24)	0.84 (0.56–1.27)	0.90 (0.61–1.33)
14 +	—	2.19 (1.15–4.17)	1.06 (0.69–1.62)	1.03 (0.72–1.46)	1.31 (1.02–1.68)

\*Adjusted for education, smoking, BMI, physical activity, diet and diseases before baseline.

**Table 2** Baseline characteristics of frequent and non-frequent drinkers according to categories of total alcohol intake.

	Alcohol intake, drinks per week							
	1–6		7–13		14–20		More than 20	
	Non-frequent	Frequent	Non-frequent	Frequent	Non-frequent	Frequent	Non-frequent	Frequent
(a) Men, characteristics*								
Subjects (n)	3508	2669	741	6981	117	3464	84	7969
Alcohol intake (median drinks per week)	3.1	4.7	8	9.4	17.6	17.9	27	30.5
Age (mean years)	56	56	56	56	56	56	56	56
Education (% lowest level)	43	37	45	31	51	31	50	32
BMI (% more than 30 kg/m <sup>2</sup> )	21	17	22	16	32	15	25	20
Smoking (% current)	39	33	42	33	44	37	60	47
Vegetable intake (% in lowest consumption group)	11	10	10	7	13	9	23	10
Fruit intake (% in lowest consumption group)	6	6	7	5	9	8	19	12
	1–6		7–13		More than 13			
	Non-frequent	Frequent	Non-frequent	Frequent	Non-frequent	Frequent		
(b) Women, characteristics†								
Subjects (number)	7417	5005	580	6326	106	5328		
Alcohol intake (median drinks per week)	2.5	4.5	7.7	8.7	18.6	20.6		
Age (mean years)	56	56	57	56	57	56		
Education (% lowest level)	38	27	40	25	42	21		
Body mass index (% more than 30 kg/m <sup>2</sup> )	19	14	17	11	18	11		
Smoking (% current)	31	26	43	29	55	41		
Vegetable intake (% in lowest consumption group)	19	16	22	14	23	15		
Fruit intake (% in lowest consumption group)	11	9	12	10	25	18		

\*In addition, 1376 men drank less than one drink per week; †4864 women drank less than one drink per week.

intake compared with non-drinkers (drinkers of more than zero but less than one drink per week) were estimated (Fig. 1). The hazard ratios of mortality were higher among non-frequent drinkers than among frequent drinkers for a weekly alcohol intake of more than one drink per week [ $P = 0.03$  (men) and  $P = 0.05$  (women) using the Wald test]. Among non-frequent drinking men, the hazard ratios were 0.61 (95% CI, 0.47–0.79), 0.61 (95% CI, 0.42–0.90), 1.11 (95% CI, 0.62–2.00) and 1.25 (95% CI, 0.70–2.24) for subjects drinking one to six, seven to 13, 14–20 and more than 20 weekly drinks, respectively. Correspondingly, the hazard ratios among frequent drinking men were 0.76 (95% CI, 0.58–0.99), 0.57 (95% CI, 0.44–0.72), 0.61 (95% CI, 0.47–0.80) and 0.83 (95% CI, 0.66–1.04). Among non-frequent drinking women, the hazard ratios were 1.05 (95% CI, 0.85–1.30), 1.31 (95% CI, 0.85–2.01) and 2.19 (95% CI, 1.15–4.17) for subjects drinking one to six, seven to 13 and more than 13 weekly drinks, respectively. Correspondingly, the hazard ratios among frequent drinking women were 0.89 (95% CI, 0.69–1.14), 0.91 (95% CI, 0.72–1.16) and 1.20 (95% CI, 0.96–1.51).

The mortality rate ratios for different combinations of quantity and frequency of alcohol intake were also estimated (Table 1a,b). For men, the lowest risk estimates was for drinking seven to 13 drinks per week distributed 5–6 days per week (0.51 95% CI: 0.36–0.73) and for drinking 14–21 drinks per week distributed on 5–6 days per week (0.52 95% CI 0.35–0.76) (Table 2a). The highest hazard ratios were obtained among men drinking on 1 day per week or more rarely; for this category the hazard ratio was 1.11 (95% CI: 0.62–2.00) for drinking 14–20 drinks per week and 1.25 (95% CI: 0.70–2.24) for drinking more than 20 drinks per week. The hazard ratio for drinking totally 21 or more drinks per week distributed on 7 days per week was 0.84 (95% CI: 0.66–1.06). For women, the lowest risk estimate was for drinking one to six drinks per week distributed on 5–6 days per week (0.72 95% CI: 0.32–1.64) and for drinking seven to 13 drinks per week distributed on 5–6 days per week (0.84 95% CI: 0.56–1.27) (Table 2a). The highest hazard ratios were obtained among women drinking on 1 day per week or more rarely; for this category the hazard ratio was 1.30 (95% CI: 0.85–2.01) for drinking seven to 13 drinks



per week and 2.19 (95% CI: 1.15–4.17) for drinking more than 13 drinks per week. The hazard ratio for drinking 14 or more drinks per week distributed on 7 days per week was 1.31 (95% CI: 1.02–1.68). Risk estimates of subjects without diseases before baseline did not differ from the estimates for all subjects (data not shown).

The following covariates were associated independently and positively with mortality for men: having a diagnosis of acute myocardial infarction, angina, other cardiovascular diseases or any chronic disease before baseline, not performing any physical activity, smoking, not eating fruit and salad, not using olive oil for cooking, having a BMI < 18 kg/m<sup>2</sup> and having school education for less than 11 years. For women, having had a diagnosis of acute myocardial infarction or any chronic disease before baseline, not performing any physical activity, smoking, not eating salad and having a BMI < 18 kg/m<sup>2</sup> were independently and positively associated with mortality.

## DISCUSSION

We found that drinking pattern influenced the relation between alcohol intake and all-cause mortality. For the same average consumption of alcohol, a non-frequent intake implied a higher risk of death than a frequent one. However, frequent heavy drinking (>20 drinks per week for men and >13 drinks per week for women) also implied an increased risk of death compared to light drinking. Our study population consisted of middle-aged men and women. This age group constitutes a high-risk population for heart diseases and it is therefore qualified for investigating how the deleterious effects of alcohol are balanced against the protecting effect, according to drinking frequency and amount of intake.

The follow-up period in this study was 6.8 years, which is a shorter period than that seen in most other epidemiological studies. This means that the information on alcohol intake given by the participants at baseline probably describes more accurately the factual behaviour of the subjects at follow-up in the present study. The combination of this relatively short follow-up period, the large number of participants and a large variation in frequency and amount of alcohol intake allows us to estimate the hazard ratios for non-frequent and frequent drinkers separately.

The finding that the association between alcohol intake and mortality depends upon drinking pattern has been suggested previously [4–7]. Although other measures of drinking patterns were used, results imply consistently a hazardous effect of drinking alcohol in large amounts per occasion. Most of these studies did not assess drinking pattern over the whole spectrum of total alcohol intake and it was difficult to differentiate between the

influence from total alcohol intake and drinking pattern. We avoided the term 'binge drinking', which in most studies is defined as drinking a minimum number of drinks per occasion, such as six or 13 [6,7], because the participants were not asked directly about occasional heavy drinking and we can therefore not comment on this with the present data. The drinking pattern in the present study was constructed by combining information on average quantity with usual drinking frequency, as has been performed in some other studies [8,10].

In the present study, covariates were distributed unequally in the two groups of drinkers for most factors and the more 'unhealthy' pattern was observed consistently among the non-frequent drinkers (Table 1). Also, Kesse *et al.* showed that dietary habits are unequally distributed on different categories of alcohol intake [15]. This underlines the importance of a thorough confounder control when addressing alcohol intake and drinking pattern as independent variables. We held information on smoking habits, physical activity, BMI, diet and school education, which provided the possibility to adjust for these potential confounders. To adjust for diet, five presumed indicators of a healthy diet were chosen: intake of fruit, vegetables, saturated fat, plant oil and fish. Adjusting for diet and physical activity reduced the difference in hazard ratios between frequent and non-frequent drinkers and hence the importance of drinking pattern. Possible confounders of our results are social factors, as high volumes of alcohol per occasion have been shown to be associated with negative social circumstances [16]. In the present study, adjustment was made for education, which is expected to correlate strongly with social status. However, more detailed information on other social factors was not accounted for.

Among light drinkers there was little difference in hazard ratios between non-frequent and frequent drinkers. Consequently, the reduced risk of death in light drinkers compared with abstainers seems to depend less on drinking pattern than suggested previously [9]. The beneficial effect of a light alcohol intake on cardiovascular disease has several plausible biological mechanisms, including an increase of serum high-density lipoprotein (HDL) [17], inhibition of platelet production, activation and aggregation [18,19] and increased fibrinolysis [20,21]. The influence of drinking pattern on these mediators has been studied in interventions with moderate to heavy drinkers, where there were no differences in lipid profile or fibrinolysis between weekend and daily drinkers [22,23]. In contrast, non-frequent drinkers had a higher degree of coronary occlusion and a decreased HDL to low-density lipoprotein ratio compared with drinkers with a more regular drinking pattern [24]. The question is whether the latter finding applies to individuals with a light to moderate alcohol intake, especially as another study has shown

that most light drinkers rarely drink daily and that most daily drinkers are not light drinkers [25].

We used information on mortality from the Civil Registration System, which is updated to 2003. In the future, it will be interesting to include information on cause-specific deaths, but because the follow-up time in the registers containing this information is much shorter than for the Civil Registration System, it is not yet possible.

The non-frequent drinking pattern compared to frequent drinking involves higher alcohol concentrations in the gastrointestinal tract and in the blood as the non-frequent drinkers consume more alcohol per drinking occasion than do frequent drinkers. This could lead to an enhancement of the harmful effects of alcohol, including alcoholic liver disease and upper gastrointestinal cancers. Wetterling *et al.* have investigated drinking patterns among alcoholics and found that the occurrence of alcohol-related disorders were more common among subjects with frequent inebriation compared with more continuous drinkers with similar life-time alcohol intake [26]. To our knowledge, the association between drinking pattern and neoplasms in the gastrointestinal tract has not been investigated. Occasional drinking of high consumptions of alcohol is probably also stronger when associated with accidents and suicide, due to increased risk-taking behaviours.

In conclusion, we found that frequency of drinking for moderate and high consumption of alcohol influenced the association between alcohol intake and mortality. At these levels, mortality was higher among non-frequent drinkers compared with frequent drinkers.

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## REFERENCES

- Poikolainen, K. (1995) Alcohol and mortality. *Journal of Clinical Epidemiology*, **48**, 455–465.
- White, I. R. (1999) The level of alcohol consumption at which all-cause mortality is least. *Journal of Clinical Epidemiology*, **52**, 967–975.
- Shaper, A. G. (1990) Alcohol and mortality: a review of prospective studies. *British Journal of Addiction*, **85**, 837–847.
- Rehm, J., Greenfield, T. K. & Rogers, J. D. (2001) Average volume of alcohol consumption, patterns of drinking, and all-cause mortality: results from the US National Alcohol Survey. *American Journal of Epidemiology*, **153**, 64–71.
- Trevisan, M., Schisterman, E., Mennotti, A., Farchi, G. & Conti, S. (2001) Drinking pattern and mortality: the Italian Risk Factor and Life Expectancy pooling project. *Annals of Epidemiology*, **11**, 312–319.
- Malyutina, S., Bobak, M., Kurilovitch, S., Gafarov, V., Simonova, G., Nikitin, Y. & Marmot, M. (2002) Relation between heavy and binge drinking and all-cause and cardiovascular mortality in Novosibirsk, Russia: a prospective cohort study. *Lancet*, **360**, 1448–1454.
- Laatikainen, T., Manninen, L., Poikolainen, K. & Vartiainen, E. (2003) Increased mortality related to heavy alcohol intake pattern. *Journal of Epidemiology and Community Health*, **57**, 379–384.
- Mukamal, K. J., Conigrave, K. M., Mittleman, M. A., Camargo, C. A. Jr, Stampfer, M. J., Willett, W. C. & Rimm, E. B. (2003) Roles of drinking pattern and type of alcohol consumed in coronary heart disease in men. *New England Journal of Medicine*, **348**, 109–118.
- Murray, R. P., Connett, J. E., Tyas, S. L., Bond, R., Ekuma, O., Silversides, C. K. & Barnes, G. E. (2002) Alcohol volume, drinking pattern, and cardiovascular disease morbidity and mortality: is there a U-shaped function? *American Journal of Epidemiology*, **155**, 242–248.
- McElduff, P. & Dobson, A. J. (1997) How much alcohol and how often? Population based case-control study of alcohol consumption and risk of a major coronary event. *British Medical Journal*, **314**, 1159–1164.
- Tjønneland, A., Gronbaek, M., Stripp, C. & Overvad, K. (1999) Wine intake and diet in a random sample of 48 763 Danish men and women. *American Journal of Clinical Nutrition*, **69**, 49–54.
- Overvad, K., Tjønneland, A., Haraldsdottir, J., Ewertz, M. & Jensen, O. M. (1991) Development of a semiquantitative food frequency questionnaire to assess food, energy and nutrient intake in Denmark. *International Journal of Epidemiology*, **20**, 900–905.
- World Health Organization (WHO) (2000) *Obesity: Preventing and Managing the Global Epidemic*. Report of a WHO consultation, no. 894. Geneva: WHO.
- Cox, D. R. (1972) Regression models and time tables. *Journal of the Royal Statistical Society*, **34**, 187–202.
- Kesse, E., Clavel-Chapelon, F., Slimani, N. & van Liere, M. (2001) Do eating habits differ according to alcohol consumption? Results of a study of the French cohort of the European Prospective Investigation into Cancer and Nutrition (E3N-EPIC). *American Journal of Clinical Nutrition*, **74**, 322–327.
- Rehm, J. & Gmel, G. (1999) Patterns of alcohol consumption and social consequences. Results from an 8-year follow-up study in Switzerland. *Addiction*, **94**, 899–912.
- Langer, R. D., Criqui, M. H. & Reed, D. M. (1992) Lipoproteins and blood pressure as biological pathways for effect of moderate alcohol consumption on coronary heart disease. *Circulation*, **85**, 910–915.
- Mikhailidis, D. P., Barradas, M. A. & Jeremy, J. Y. (1990) The effect of ethanol on platelet function and vascular prostanooids. *Alcohol*, **7**, 171–180.
- Mikhailidis, D. P., Jenkins, W. J., Barradas, M. A., Jeremy, J. Y. & Dandonna, P. (1986) Platelet function defects in chronic alcoholism. *British Medical Journal (Clinical Research Edition)*, **293**, 715–718.

20. Krobot, K., Hense, H. W., Cremer, P., Eberle, E. & Keil, U. (1992) Determinants of plasma fibrinogen: relation to body weight, waist-to-hip ratio, smoking, alcohol, age, and sex. Results from the second MONICA Augsburg survey 1989–90. *Arteriosclerosis and Thrombosis*, **12**, 780–788.
21. Brunner, E. J., Marmot, M. G., White, I. R., O'Brien, J. R., Etherington, M. D., Slavin, B. M., Kearney, E. M. & Smith, G. D. (1993) Gender and employment grade differences in blood cholesterol, apolipoproteins and haemostatic factors in the Whitehall II study. *Atherosclerosis*, **102**, 195–207.
22. Rakic, V., Puddey, I. B., Dimmitt, S. B., Burke, V. & Beilin, L. J. (1998) A controlled trial of the effects of pattern of alcohol intake on serum lipid levels in regular drinkers. *Atherosclerosis*, **137**, 243–252.
23. Dimmitt, S. B., Rakic, V., Puddey, I. B., Baker, R., Oosttryck, R., Adams, M. J., Chesterman, C. N., Burke, V. & Beilin, L. J. (1998) The effects of alcohol on coagulation and fibrinolytic factors: a controlled trial. *Blood Coagulation and Fibrinolysis*, **9**, 39–45.
24. Gruchow, H. W., Hoffmann, R. G., Anderson, A. J. & Barboriak, J. J. (1982) Effects of drinking patterns on the relationship between alcohol and coronary occlusion. *Atherosclerosis*, **43**, 393–404.
25. Knupfer, G. (1987) Drinking for health: the daily light drinker fiction. *British Journal of Addiction*, **82**, 547–555.
26. Wetterling, T., Veltrup, C., Driessen, M. & John, U. (1999) Drinking pattern and alcohol-related medical disorders. *Alcohol and Alcoholism*, **34**, 330–336.